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An innovative approach: introducing novel sheep paternity testing panels

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Abstract: The aim of this research was to create various microsatellite paternity test panels that are efficient, cost-effective, simple, and accessible for paternity assessments in the Eşme sheep breed in Western Anatolia. The study included 2432 animals from 16 farms, consisting of 147 rams and 2285 lambs. Seventeen microsatellite markers were utilized in the study to develop these test panels, prioritizing markers with high polymorphic information content (PIC) and probability of exclusion (PE) values. By combining markers with varying PIC and PE values, 16 distinct paternity test panels were established. The study revealed mean values for various parameters of the microsatellites used, with the lowest and highest PE values observed at specific loci. The combined probability of exclusion for the test panels ranged from 0.9079844 to 0.9999998. Panels containing seven or more microsatellites with an exclusion probability above 0.999 were identified as suitable for paternity tests in the Eşme sheep breed. These findings have significantly advanced the development of efficient and cost-effective paternity tests in animal breeding programs.

Key words: Exclusion probability, extensive breeding, parentage verification, paternity test, microsatellites, small ruminants

1. Introduction

The livestock sector in Türkiye holds a significant position and potential among agricultural activities [1–3]. Türkiye, considered one of the most significant domestic breeding centers, also provides essential infrastructure for animal husbandry because of its geographical location and climatic conditions [4–7]. Sheep constitute 78.9% of the approximately 57 million sheep and goats in Türkiye.[1] In this context, Türkiye emerges as a reservoir of an important livestock heritage, being home to 41 different sheep breeds.[2]

Sheep breeding activities, which hold significant cultural importance in Türkiye, are commonly carried out under extensive conditions [2,8,9]. In Türkiye, it is relatively challenging to maintain yield and pedigree records in sheep breeding systems, which are primarily family-owned. In 2005, the Ministry of Agriculture and Forestry General Directorate of Agricultural Research and Policies introduced the National Genetic Improvement Project for Small Ruminants at Breeders' Conditions nationwide. Thanks to this breeding initiative, farms participating in the program systematically maintain their yield records and adhere to controlled mating programs as closely as possible. However, the absence of the necessary infrastructure for controlled mating on some farms occasionally leads to inaccuracies in processing pedigree records. Moreover, it is almost impossible to obtain pedigrees from these farms due to the widespread practice of free mating on farms not affiliated with any breeding program.

Ensuring the provision of accurate information about ancestors in selection programs is crucial for estimating genetic parameters with precision. Therefore, accurate and reliable pedigree records maintained at livestock farms play a crucial role in ensuring the estimation of these parameters. It is possible to identify two types of pedigree errors on farms: incorrect pedigree information and incomplete (unknown) pedigree information [10]. The most critical of these errors is incorrect pedigree information. Inaccurate pedigree information leads to biased estimation of genetic parameters and breeding values in animal breeding programs, which, in turn, negatively affects genetic progress [11–13].

The most critical pedigree inaccuracies occur in situations such as large herd size, extensive breeding, and uncontrolled mating [14,15]. The utilization of molecular

[1]Food and Agriculture Organization of the United Nations. Crops and livestock products [online]. Website https://www.fao.org/faostat/en/#data/QCL [accessed 20 January 2024]

[2]Food and Agriculture Organization of the United Nations. Breed data sheet [online]. Website https://www.fao.org/dad-is/browse-by-country-and-species/en/ [accessed 20 January 2024]

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genetic markers has enabled the elimination of missing pedigree information in enterprise records and the verification of pedigree information, thereby preventing errors [16–18]. Microsatellites and SNPs are the most commonly utilized genome-wide markers in paternity testing to confirm pedigree records among molecular genetic markers [16,19–21].

Breeding programs must seek solutions that can provide reliable pedigree information, especially in livestock activities such as sheep and goat breeding in extensive conditions. It is also important that these solutions are inexpensive, easy to implement, and accessible. Numerous studies have demonstrated that paternity test panels utilizing microsatellites in paternity tests for accurate pedigree records are effective, cost-effective, easy to use, and accessible [15,22–27].

In 2007, a breeding program was initiated to enhance fertility and lamb growth traits in Eşme sheep, a native breed in Western Anatolia. As a result of the scientific research activities conducted in this field, the Eşme breed was officially recognized as a national breed in 2020 [28–31].

The objective of the present study was to establish multiple microsatellite paternity test panels suitable for efficient, cost-effective, simple, and accessible paternity assessments in the Eşme sheep breed, which has gained significance in Western Anatolia.

2. Materials and methods

2.1. Animal material

A total of 2432 sheep from the Eşme breed were included in the study, comprising 2285 offspring from 16 breeder farms, along with 147 rams involved in a controlled mating program on these farms. Blood was collected from the rams during the breeding season and from the lambs during the lambing period. The blood was drawn from the jugular vein into 5-mL tubes containing K3EDTA using the appropriate technique. The collected DNA was stored at –20 °C until molecular genetic analyses were performed.

2.2. DNA isolation, polymerase chain reaction (PCR), and fragment analysis

A commercial DNA isolation kit (Applied Biological Materials Inc., Canada) was used to extract genomic DNA. After the DNA was isolated, its quality and quantity were assessed using a NanoDrop 2000 spectrophotometer manufactured by Thermo Scientific, USA.

For this research, 17 microsatellite markers recommended by the FAO [32] were used to create paternity test panels. Microsatellites with similar allele sizes were labeled with different fluorescent dyes (D2, D3, and D4) recognized by the Beckman GeXP device (Table 1). Two multiplex groups (Table 2) were then formed by considering the allele sizes and fluorescent markers of the microsatellites used in the study to enable the PCR of multiple microsatellites in the same well.

Table 1. Details of considered microsatellite loci [32].

Multiplex group	Primer name (Accession no.)	Allelic range (Chr. no.)	Label	Primer sequence
M1	BM8125 (G18475)	110–130 (OAR17)	D3	CTCTATCTGTGGAAAAGGTGGG
				GGGGGTTAGACTTCAACATACG
	CSRD0247 (---)	209–261 (OAR14)	D3	GGACTTGCCAGAACTCTGCAAT
				CACTGTGGTTTGTATTAGTCAGG
	HSC (M90759)	267–301 (OAR20)	D2	CTGCCAATGCAGAGACACAAGA
				GTCTGTCTCCTGTCTTGTCATC
	BM1329 (G18422)	145–161 (OAR6)	D2	TTGTTTAGGCAAGTCCAAAGTC
				AACACCGCAGCTTCATCC
	MAF214 (M88160)	174–282 (OAR16)	D4	GGGTGATCTTAGGGAGGTTTTGGAGG
				AATGCAGGAGATCTGAGGCAGGGACG
	McM0527 (L34277)	165–179 (OAR5)	D3	GTCCATTGCCTCAAATCAATTC
				AAACCACTTGACTACTCCCCAA
	OarFCB128 (L01532)	96–130 (OAR2)	D2	ATTAAAGCATCTTCTCTTTATTTCCTCGC
				CAGCTGAGCAACTAAGACATACATGCG
	OarJMP29 (U30893)	96–150 (OAR24)	D4	GTATACACGTGGACACCGCTTTGTAC
				GAAGTGGCAAGATTCAGAGGGGAAG
M2	BM1818 (G18391)	258–270 (23)	D4	AGCTGGGAATATAACCAAAGG
				AGTGCTTTCAAGGTCCATGC
	D5S2 (Z22743.1)	190–210 (---)	D4	TACTCGTAGGGCAGGCTGCCTG
				GAGACCTCAGGGTTGGTGATCAG
	INRA0132 (EF507691.1)	152–172 (20)	D4	AACATTTCAGCTGATGGTGGC
				TTCTGTTTTGAGTGGTAAGCTG
	INRA0023 (X67830)	195–225 (3)	D3	GAGTAGAGCTACAAGATAAACTTC
				TAACTACAGGGTGTTAGATGAACTC

Table 1. (Continued.)

	OarAE0129 (L11051)	135–165 (5)	D2	AATCCAGTGTGTGAAAGACTAATCCAG
				GTAGATCAAGATATAGAATATTTTTCAACACC
	OarCP34 (U15699)	112–130 (OAR3)	D4	GCTGAACAATGTGATATGTTCAGG
				GGGACAATACTGTCTTAGATGCTGC
	OarFCB193 (L01533)	96–136 (OAR11)	D3	TTCATCTCAGACTGGGATTCAGAAAGGC
				GCTTGGAAATAACCCTCCTGCATCCC
	OarFCB20 (L20004)	92–118 (OAR2)	D2	AAATGTGTTTAAGATTCCATACAGTG
				GGAAAACCCCCATATATACCTATAC
	OarFCB304 (L01535)	148–190 (OAR19)	D3	CCCTAGGAGCTTTCAATAAAGAATCGG
				CGCTGCTGTCAACTGGGTCAGGG

Table 2. Touch-down PCR conditions.

Multiplex group	First denaturation	Denaturation	Annealing	Extension	Cycle	Final extension
M1	95 °C (5 min)	95 °C (40 s)	60–50 °C (40 s)	72 °C (1 min)	34	72 °C (10 min)
M2	95 °C (5 min)	95 °C (40 s)	63–54 °C (40 s)	72 °C (1 min)	30	72 °C (10 min)

The PCR was conducted in a total volume of 25 µL, using approximately 50 ng of DNA. The PCR master mix was composed of 0.10 µM primer, 0.20 mM dNTPs, 2.0 mM $MgCl_2$, 1X PCR buffer, and 1 unit of Taq DNA polymerase. Since the microsatellites used in the study had different annealing temperatures, the touch-down PCR method [33] was employed (Table 2).

Fragment analysis of fluorescently labeled microsatellites was performed on a Beckman Coulter GeXP genetic analyzer in accordance with the manufacturer's recommendations.

2.3. Setting up paternity test panels

The 17 microsatellite markers used in the study were ranked from high to low based on polymorphic information content (PIC) and probability of exclusion (PE) values. Microsatellites with lower values were then added one by one to the microsatellite marker with the highest PIC and PE values to form 16 different paternity test panels (Table 3).

2.4. Statistical analysis

GenALEX genetic analysis software [34] was used to define allele number (Na), effective allele number (Ne), observed heterozygosity (Ho) and expected heterozygosity (He) values, and Hardy–Weinberg equilibrium. Additionally, PIC, PE, probability of identity (PI), probability of combined exclusion (CPE), combined probability of identity (CPI), mean proportion of genotyped individuals (GR), and null allele frequency (F(Null)) values were obtained using the program CERVUS[3] [35–37]. Formulas for the paternity test statistics are presented in the supplementary file (S1). The PROC CORR procedure in the statistical software package SAS [38] was used to analyze the phenotypic correlations among PIC, PE, and PI values.

3. Results

Table 4 presents the molecular genetic variability findings related to paternity testing for each microsatellite used in the investigation.

Across the 17 microsatellites analyzed, a total of 481 alleles were observed. Notably, the MAF214 locus exhibited the highest number of alleles (57), while the OARCP34 locus exhibited the lowest number of alleles (19). The PIC values derived from the examined microsatellites exhibited a range exceeding 0.75, with an overall mean value of 0.85 for this parameter. The average values calculated for both Ho and He across all examined loci were 0.72 and 0.86, respectively. The results of the χ^2 test for Hardy–Weinberg equilibrium indicate that the allele distributions of all microsatellites used in the study were not in this equilibrium. The highest PE value, which is a crucial parameter in resolving parentage disputes in paternity test studies, was observed at the CSRD247 locus. The PI values, which are used to determine the likelihood of random genetic profile matching between pairs of individuals examined in the study, ranged from 1.29E–02 to 6.51E–02. Additionally, null allele frequencies obtained for all investigated microsatellites were less than 0.20.

Phenotypic correlation coefficients among PIC, PE, and PI in the study are provided in Table 5.

When examining Table 5, a high positive correlation coefficient is found between PIC and PE, while a high negative correlation coefficient is found between PE and PI.

Table 6 illustrates the statistical outcomes of paternity test panels constructed using varying numbers of microsatellites, based on their PIC and PE values.

[3]Marshall TC. (1998/2006). Cervus 3.0 [online]. Website https://www.fieldgenetics.com/ [accessed 02 July 2008]

Table 3. Paternity test panels (P) created according to the individual PIC and PE values of microsatellites.

Microsatellite			Panels															
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
CSRD0247	PIC	0.91	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	PE	0.71																
HSC	PIC	0.90	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	PE	0.68																
INRA0023	PIC	0.90		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	PE	0.68																
MAF214	PIC	0.88			*	*	*	*	*	*	*	*	*	*	*	*	*	*
	PE	0.65																
BM8125	PIC	0.87				*	*	*	*	*	*	*	*	*	*	*	*	*
	PE	0.62																
BM1818	PIC	0.87					*	*	*	*	*	*	*	*	*	*	*	*
	PE	0.63																
OarFCB20	PIC	0.86						*	*	*	*	*	*	*	*	*	*	*
	PE	0.61																
OARJMP29	PIC	0.86							*	*	*	*	*	*	*	*	*	*
	PE	0.60																
BM1329	PIC	0.86								*	*	*	*	*	*	*	*	*
	PE	0.60																
INRA0132	PIC	0.86									*	*	*	*	*	*	*	*
	PE	0.60																
OARFCB128	PIC	0.85										*	*	*	*	*	*	*
	PE	0.58																
OarAE0129	PIC	0.84											*	*	*	*	*	*
	PE	0.56																
MCM0527	PIC	0.83												*	*	*	*	*
	PE	0.54																
OarFCB304	PIC	0.83													*	*	*	*
	PE	0.55																
OarFCB193	PIC	0.80														*	*	*
	PE	0.49																
OarCP34	PIC	0.78															*	*
	PE	0.45																
D5S2	PIC	0.78																*
	PE	0.45																
No. of microsatellites in the panels			2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17

PIC: polymorphic information content, PE: probability of exclusion, * microsatellites used in the panel.

Table 4. Genetic variability and paternity analysis parameters of microsatellites.

Loci	Na	Ne	Ho	He	PIC	PE	PI	HWE	F(Null)
CSRD0247	39	11.86	0.79	0.92	0.91	0.713	1.29E-02	***	0.071
INRA0023	23	10.23	0.66	0.90	0.90	0.682	1.59E-02	***	0.159
HSC	27	10.39	0.70	0.90	0.90	0.680	1.64E-02	***	0.130
MAF214	57	9.03	0.74	0.89	0.88	0.647	2.08E-02	***	0.096
BM1818	31	8.18	0.73	0.88	0.87	0.625	2.31E-02	***	0.085
BM8125	28	8.42	0.71	0.88	0.87	0.623	2.39E-02	***	0.112
OarFCB20	23	7.95	0.80	0.87	0.86	0.608	2.66E-02	***	0.035
BM1329	35	7.56	0.59	0.87	0.86	0.601	2.73E-02	***	0.184
INRA0132	20	7.58	0.74	0.87	0.86	0.597	2.78E-02	***	0.074
OARJMP29	34	7.72	0.72	0.87	0.86	0.598	2.83E-02	***	0.095
OARFCB128	27	7.50	0.71	0.87	0.85	0.579	3.15E-02	***	0.097

Table 4. (Continued.)

OarAE0129	23	6.62	0.67	0.85	0.84	0.556	3.59E-02	***	0.118
OarFCB304	28	6.41	0.77	0.84	0.83	0.547	3.88E-02	***	0.039
MCM0527	21	6.51	0.67	0.85	0.83	0.541	3.94E-02	***	0.117
OarFCB193	26	5.28	0.80	0.81	0.80	0.493	4.89E-02	***	–0.007
OarCP34	19	5.09	0.83	0.80	0.78	0.448	6.38E-02	***	–0.024
D5S2	20	5.03	0.61	0.80	0.78	0.446	6.51E-02	***	0.138
Overall Mean	**28.29**	**7.73**	**0.72**	**0.86**	**0.85**				

Na: number of alleles, Ne: number of effective alleles, Ho: observed heterozygosity, He: expected heterozygosity, PIC: polymorphic information content, PE: probability of exclusion, PI: probability of identity, HWE: Hardy-Weinberg Equilibrium (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$), F(Null): null allele frequency.

Table 5. Phenotypic correlation coefficients among PIC, PE, and PI.

	PIC	**PE**
PE	0.998***	
PI	–0.967***	-0.968***

PIC: polymorphic information content, PE: probability of exclusion, PI: probability of identity, ***: $p < 0.001$.

The assessment of paternity test panels involved an analysis of key parameters, including the mean number of alleles (MNa), mean expected heterozygosity (MHe), mean polymorphic information content (MPIC), CPE, and CPI. Upon scrutiny, Panel 4 exhibited the highest MNa value, while Panel 1 showcased the highest MHe and MPIC values. Noteworthy is the attainment of a 99.99% CPE value for Panel 8. The trajectory of CPE values demonstrated a rapid escalation until Panel 4, followed by a moderate incline from Panel 5 onward. Variations in the CPI values ranged from 2.11E–04 (Panel 1) to 7.73E–27 (Panel 16), with Panel 16 representing the peak.

4. Discussion

Upon analyzing the MNa, Ho, He, and PIC values obtained from the study, it is evident that the microsatellites used demonstrate a significantly high level of polymorphism. Particularly noteworthy is the substantially higher MNa value obtained in comparison to several prior studies on the subject [39–44]. Conversely, the results related to Ho, He, and PIC values were lower compared to those in some studies [45–47] and higher than those in others [39,41,48,49]. The observed disparities in genetic polymorphism statistics compared to the literature are presumed to arise from variations in both breed characteristics and the microsatellites used. The elevated PIC value, which is crucial in formulating paternity test panels, observed in the present study notably enhanced the efficacy of the constructed paternity test panels within the study's framework. The obtained χ^2 test results show that the allele distributions of all microsatellite loci are not in Hardy–Weinberg equilibrium. In previous studies [39–43], similar situations to the findings regarding compliance with Hardy–Weinberg equilibrium have been reported. This can be considered a typical finding considering the selection studies conducted in the populations under investigation.

The individual PE values of microsatellites, a critical parameter for designing effective paternity test panels and ensuring their reliability, were derived in the present study. Notably, these values exceed those documented in prior research on the subject [15,25,50,51]. The correlation between PE and PI is significantly important in the context of paternity testing. Upon analyzing this relationship, a strong negative correlation of –0.968 is observed. Although no definitive mathematical relationship exists, a lower PI value corresponds to a higher PE value. Conversely, a higher PI value indicates greater genetic similarity among the individuals under study, thereby complicating the exclusion of nonpaternal candidates in paternity tests. When analyzing the PI values obtained in the study, it is observed that these values fall within the ranges outlined in the literature [23–25,51,52].

On the other hand, the null allele frequencies determined for all microsatellites used in the study were lower than the critical threshold (0.20) defined by Dakin and Avise [53]. Given the parameters of PIC, PE, and PI and the null allele frequencies derived from the study, it was ascertained that all microsatellites used can be reliably incorporated into paternity test panels. Upon analyzing the PIC and PE values obtained from the microsatellites used, a significant positive correlation (0.998) between these parameters was observed. Consequently, these metrics were prioritized as primary criteria in formulating paternity test panels within the study. It is noteworthy that all CPI values obtained for the constructed paternity test panels fall within the range reported by Waits [54]. This observation suggests dissimilarity in genetic characteristics

Table 6. Genetic variability and paternity analysis parameters for paternity test panels.

Panels	NMP	MNa	MHe	MPIC	CPE	CPI	GR (%)
1	2	33.00	0.91	0.9033	0.9079844	2.11E-04	92.46
2	3	29.67	0.91	0.9008	0.9707122	3.35E-06	90.80
3	4	36.50	0.90	0.8958	0.9896706	6.96E-08	92.33
4	5	34.80	0.90	0.8909	0.9961068	1.67E-09	92.97
5	6	34.17	0.89	0.8874	0.9985402	3.86E-11	92.94
6	7	32.57	0.89	0.8839	0.9994286	1.03E-12	92.57
7	8	32.75	0.89	0.8808	0.9997701	2.91E-14	93.16
8	9	33.00	0.89	0.8783	0.9999082	7.94E-16	92.87
9	10	31.70	0.89	0.8762	0.9999630	2.21E-17	93.30
10	11	31.27	0.88	0.8741	0.9999845	6.94E-19	93.54
11	12	30.58	0.88	0.8709	0.9999931	2.49E-20	93.35
12	13	29.85	0.88	0.8679	0.9999968	9.81E-22	93.57
13	14	29.71	0.88	0.8652	0.9999986	3.81E-23	93.83
14	15	29.47	0.87	0.8607	0.9999993	1.86E-24	94.07
15	16	28.81	0.87	0.8556	0.9999996	1.19E-25	94.29
16	17	28.29	0.86	0.8509	0.9999998	7.73E-27	94.34

NMP: number of microsatellites in the panel, MNa: mean number of alleles, MHe: mean expected heterozygosity, MPIC: mean polymorphic information content, CPE: combined probability of exclusion, CPI: combined probability of identity, GR: mean proportion of the genotyping individuals.

within the study cohort, thereby increasing the likelihood of higher exclusion probabilities.

The minimum CPE value reported in the literature to accurately identify the true father is 0.999 [18,55–57].

5. Conclusion

Upon scrutinizing the CPE values across the 16 distinct paternity test panels devised within the study, it is discerned that Panels 1–5 exhibit inadequately low exclusion probability values, making them unsuitable for use in paternity testing endeavors. However, panels containing 7 or more microsatellites had a PE value above 0.999, making them suitable for use in paternity tests in Eşme sheep. However, cost, ease of analysis, and reliability are crucial factors in paternity tests. In this context, Panel 6, which consists of 7 microsatellites, stands out. With the use of this panel, it was revealed that a lower-cost, reliable, and easier-to-analyze test can be performed compared to other panels.

Accurate and reliable acquisition of genetic parameters is of paramount importance in animal breeding programs. Instances of errors in pedigree records, which are common in animal breeding, can significantly compromise the accuracy of genetic parameter estimations. This, in turn, undermines the efficacy of selection processes, potentially hindering the realization of desired genetic progress. Consequently, the increasing significance of paternity tests within breeding programs is evident, serving as crucial tools to mitigate inaccuracies and enhance the effectiveness of genetic management strategies. The most tangible problem of the breeding programs conducted in small ruminant breeding models, such as in Türkiye, where small ruminant breeding is primarily done under extensive conditions, is ensuring the accuracy of the results reported in hand-mating practices and correctly identifying the parents. In the present study, which was conducted on a very large population, the effectiveness of the microsatellites used was demonstrated, and recommendations for paternity test panels with fast, economical, and high accuracy were presented. The valuable findings obtained will make a significant contribution to solving the paternity test issue under breeders' conditions in comprehensive projects such as the National Genetic Improvement Project for Small Ruminants at Breeders' Conditions conducted by the Ministry of Agriculture and Forestry.

Informed consent

This study was approved by the Aydın Adnan Menderes University Animal Experiments Local Ethics Committee (Approval no: 124-HEK/2009/53).

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Conflict of interest

The authors declare that there are no conflicts of interest.

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Supplementary Documents

Allele number (Na), effective allele number (Ne), observed heterozygosity (Ho), expected heterozygosity (He) and Hardy-Weinberg equilibrium (HWE) were obtained using the GenALEX analysis program [1], following the equations provided in the literature [2-4].

Mathematical formulas for Na, Ne, Ho, He and chi-square for HWE

$$Na = \frac{\sum Na_i}{r}$$

$$Ne = \frac{1}{\sum X_i^2}$$

$$Ho = \sum \frac{N_{ij}}{N}$$

$$He = 1 - \sum p_i^2$$

In the formula Na_i = total number of alleles at locus i, X_i = mean number of alleles at locus *i*, *r* = total number of loci studied, *Ho* = observed heterozygosity, N_{ij} = number of heterozygous individuals, *N* = total number of individuals analysed, *He*=expected heterozygosity, P_i=allelic frequency.

Polymorphic information content (PIC) values were calculated using the formula reported by Botstein et al. [5] with the Cervus program [6-9].

$$PIC = 1 - \sum_{i=1}^{n} p_i^2 - 2\left[\sum_{n=1}^{n=1} \sum_{j=i+1}^{n} p_i^2 p_j^2\right]$$

In the formula *PIC*=polymorphic information content, p_i=number of alleles at i^{th} locus, *n*=number of alleles.
Probability of exclusion (PE), combined probabilities of exclusion (CPE) were calculated in the Cervus programme [6-9] using formulae described in the literature [10].

$$P_E = h^2 \times \left(1 - 2hH^2\right)$$

$$CP_E = 1 - \prod_{i=1}^{n} (1 - P_{Ei})$$

In the formula, *PE* = exclusion probability, *h* = number of heterozygotes, *H* = number of homozygotes, *CPE* = combined exclusion probability.

The probability of identity (PI) was calculated in the Cervus programme [6-9] using the formulae described in the literature [11].

$$PI = \sum p_i^4 + \sum \sum \left(2p_i 2p_j\right)^2$$

where p_i and p_j are the frequencies of the i^{th} and j^{th} alleles and $i \neq j$

Pearson correlation coefficient was calculated using the following formula in SAS [12] statistical package programme.

$$p_{XY} = \frac{cov(XY)}{\sigma_X \sigma_Y}$$

Where *cov* is the covariance, σ is the standard deviation of *X*, and σ is standard deviation of *Y*."

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