ijlpr 2022; doi 10.22376/ijpbs/lpr.2022.12.6.L86-94

International Journal of Life science and Pharma Research

ISSN 2250-0480

Research Article

Molecular Genetics

Assessment of Microsatellite Markers for Parentage Testing in Jersey Crossbred Cattle in Tamil Nadu

***Hepsibha P[1], Karthickeyan S M K[2] and Judia Harriet Sumathy V[3]**

[1,3] *Women's Christian College, University of Madras, Chennai, Tamil Nadu, India*

[2] *Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University Chennai, Tamil Nadu, India*

Abstract: A study was aimed to evaluate a set of polymorphic microsatellite markers for their efficiency in testing parentage in Jersey crossbred cattle available in Tamil Nadu. The objective of validation was based on the criteria such as polymorphism information content, various genetic diversity parameters and the ability of markers to exclude the wrong parent. A total of 21 microsatellite markers were included to verify the parentage in 24 Jersey crossbred trios (comprising 24 dams, 24 progenies and 2 sires). The microsatellite loci were amplified using fluorescently labelled primers by multiplex PCR and fragment analysis was done through capillary electrophoresis using automated DNA analyzer. Statistical analyses were done using Pop gene version 1.31, CERVUS 3.0.7 and GENALEX 6.503 software programs. The number of alleles for the markers utilized in the study ranged from 4 (ILSTS11) to 12 (INRA23 and TGLA122) with an overall mean of 8.0952 ± 0.47 alleles per locus. The markers also exhibited high expected heterozygosity (H_e) and polymorphism information content (PIC) ranging from 0.6362 (ETH3) to 0.8629 (SPS115) with a mean of 0.7681 ± 0.013 and 0.592(ETH3) to 0.83(CSSM66) with a mean of 0.7256±0.014 respectively, signifying them to be highly informative. Low overall probability of identity (PI) of 0.0943 ± 0.008 and high overall probability of exclusion (PE) of 0.9619±0.02 with the increasing locus combinations were observed. The probability of exclusion was cent per cent (PE=1.0000) when a combination of 8 markers were used with one known parent and a combination of 12 markers when excluding a putative pair, suggesting the efficacy and suitability of the markers used in the study for parentage testing on Jersey crossbreds of Tamil Nadu.

Keywords: Microsatellite Markers, Jersey Crossbred Cattle, Parentage Testing, Probability of Exclusion (PE).

***Corresponding Author**

Hepsibha P , Women's Christian College, University of Madras, Chennai, Tamil Nadu, India

Received On 19 September 2022

Revised On 29 September 2022

Accepted On 01 October 2022

Published On 01 November 2022

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Citation Hepsibha P , Karthickeyan S M K and Judia Harriet Sumathy V , Assessment of Microsatellite Markers for Parentage Testing in Jersey Crossbred Cattle in Tamil Nadu.(2022).Int. J. Life Sci. Pharma Res.12(6), L86-94 http://dx.doi.org/10.22376/ijpbs/lpr.2022.12.6.L86-94

1. INTRODUCTION

Parentage testing in cattle is an important aspect today to implement efficient breeding programs by selective reproduction through proven bulls. Failure to record the correct parentage can cause bias in sire evaluation, by introducing errors in estimation of heritabilities and breeding values [1]. Parentage testing relies on the principle that an individual will inherit one copy of its genes from its mother and other from its father[2] . Therefore, if a particular marker size (called an allele) is present in the calf, but absent in both of the nominated parents, then the parents must be excluded from the calf's pedigree. Bovine breeding act was promulgated to regulate bovine breeding activities and for improving production of bovines as per the Animal Husbandry Policy note (2020-21)[3]. Majority of the cattle reared by the farmers in Tamil Nadu comprise of high milk yielding crossbred cattle with exotic germplasm like Jersey and Holstein Friesian (HF). While Jersey cattle is the breed of choice in the plains and, Holstein Friesian in the hilly areas where the climate is conducive for rearing this breed. Though there are much literature on parentage testing in Holstein Friesian cattle, there is scarce information on Jersey crossbreds especially in India[4,7]. So this study targeted on validation of a set of microsatellite markers and their efficiency in identifying parentage in Jersey crossbred population using various diversity parameters and probability measures[8]. Different methodologies[4,5] of parentage verification in general have been used since 1940's, starting from blood typing[9] to automated analysis of SNPs[10] of which, molecular markers are of great interest to determine the degree of genetic relatedness between animals, making parentage verification and individual identification much easier[11] . In 1990's, microsatellites were highly exploited as they exhibited high degree of polymorphism and even with the rise of next generation sequencing microsatellite markers still continue to be the gold standard for parentage control in most breeding programs of cattle. Microsatellites (sometimes referred to as Simple Sequence Repeats, or SSR) are repetitive DNA sequences made up of blocks of 1 to 6 nucleotides that are repeated up to 60 times[12]. The markers were also recommended by the International Society for Animal Genetics (ISAG) - Food and agriculture organization (FAO) Advisory Group on Animal Genetic Diversity in 2011)[13] to be used for paternity exclusion tests due to their extensive utility in parentage control in several species, high abundance, informativeness, and relatively low costs[14]. Accurate allele sizes of the microsatellite loci can be determined by using cutting-edge techniques like capillary electrophoresis[15], which has numerous benefits over conventional separation techniques like PAGE, such as separation efficiency, short analysis time, low sample and solvent consumption, low operating costs, and lower matrix effects[16]. Using fluorescent dye-labelled primers with automatic capillary electrophoresis is one of the most popular high-sizing precision method termed "Fragment analysis" to assess the polymorphisms of the loci [17-19] . With this background, this study has utilized 21 fluorescently labelled microsatellite markers selected based on FAO recommendation (FAO Guidelines, 2011)[13] as well as published literature to verify the parentage in Jersey crossbred cattle present in Tamil Nadu, South India. Their efficiency was also measured using various statistical methods and software. Various genetic parameters were estimated to establish a parentage test for Jersey crossbred cattle viz., allelic richness, heterozygosity (H_e), PIC and multi-loci combination tests for probabilities of identity and exclusion.

2. MATERIALS AND METHODS

2.1 Genomic DNA Isolation

Genomic DNA was isolated from blood samples of 24 Jersey crossbred trios (comprising 24 dams, 24 progenies and 2 sires) received from various farms and milk procuring units of state of Tamil Nadu, for parentage verification at the Department of Animal Genetics and Breeding, Madras Veterinary College (Figure1). Genomic DNA from blood was isolated by modified phenol-chloroform method as suggested by Sambrook *et al.*,1989[20] using DNAzol (Invitrogen™) instead of proteinase-k. Genomic DNA of the sires were isolated from the frozen semen straws. Initially the samples were treated with sperm lysis buffer[21] (comprising of 20mM Tris Hcl, 20mM EDTA, 200mM Nacl, 4% SDS and, 2% beta-mercaptoethanol) and 500µl of DNAzol. The remaining steps were as per phenol-chloroform method[20]. The genomic DNA samples were quantitatively and qualitatively checked using Nanodrop One C spectrophotometer (Thermo Scientific, USA) at absorbance A260/280 ratio and A260/230 ratio.

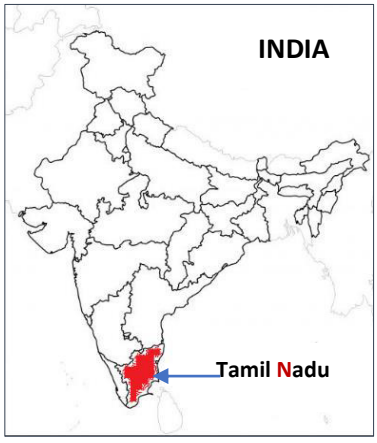

Fig 1. Geographical location of the Jersey Crossbred cattle population of the study in Tamil Nadu, India

2.2 *Microsatellite markers and multiplex PCR*

Twenty-one microsatellite markers were selected based on FAO Guidelines, 2011[13] as well as from reported literature[22-24] . The chromosomal numbers of the markers located in and their allelic size ranges are mentioned in Table 1 as referred from the literature. The primers for these markers were synthesized with fluorescent labelling at their 5' end using FAM-BLUE, HEX-green, TET-green and TAMRA-black dyes (Table1). The markers were grouped into five multiplex panels based on their annealing temperature, product size (bp) and fluorescent labels to perform multiplex PCR. The PCR reaction mix (25µl) consisted of colorless master mix (AMPLIQON) of 2X concentration, panel of 5 primer sets (concentration of each varying from 4 to 7 picomoles), template DNA (50ng) and nuclease free water. The PCR conditions had initial denaturation at 95°C for 5 minutes, then 35 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C-61.5°C (varies for each panel) for 30 seconds and extension at 72°C for 40 seconds and then with a final extension of 72°C for 5 minutes.

Table1: Chromosomal locations, allelic size ranges(bp) and fluorescent labels of the markers utilized in the study.

Markers	Chromosomal number	Allele size range (bp)	5'end Fluorescent labelling
TGLA126	20	116-135	FAM
ILSTS006	7	272-309	FAM
INRA23	3	194-225	HEX
INRA63	18	167-189	TAMRA
HEL9	8	141-173	FAM
BM1824	1	181-200	TET
ILSTS11	14	232-320	HEX
TGLA227	18	76-105	TAMRA
TGLA122	21	206-240	TET
ETH10	5	171-209	HEX
CSSM66	14	171-209	FAM
CSRM60	10	79-130	TAMRA
BM2113	2	125-150	HEX
ETH3	19	96-150	TET
SPS115	15	234-258	TET
BM1818	23	230-280	TET
ETH152	5	180-220	TAMRA
MM12	9	101-145	TAMRA
TGLA53	16	143-191	FAM
INRA5	12	136-164	FAM
ILSTS33	12	120-175	TAMRA

2.3 *Fragment analysis*

The PCR products (amplicons) were diluted in the ratio of 1: 60 and combined with Hi-di formamide and GS500liz size standard. The amplicons were then separated through capillary electrophoresis (CE) using ABI's 3730xl DNA analyzer and were resolved by comparing with the size standard. Gene Mapper® software as well as Peak Scanner 2 software were used for DNA sizing and allele calling.

3. STATISTICAL ANALYSIS

Popgene version 1.31[25], was utilized to study the diversity metrics *viz*., observed number of alleles (N_a), effective number of alleles (N_e), allele frequency, observed heterozygosity value (H_o), and expected heterozygosity value (H_e) of all loci from the genotypes. CERVUS 3.0[26] was used to evaluate the markers' polymorphism information content (PIC) [27]. The probabilities of identity (PI) and exclusion (PE) based on allele frequencies were also estimated using the GenAlEx 6.503 algorithm[28].

4. RESULTS AND DISCUSSION

4.1 *Genomic DNA Isolation*

The concentration of genomic DNA isolated from blood and semen samples ranged between 500ng and1000ng and the purity of the DNA was 1.8 at absorbance 260/280 and between 2 and 2.2 at absorbance ratio 260/230. Similar yields of genomic DNA using phenol chloroform extraction procedure had been reported previously by many authors [29-32].

4.2 *Multiplex PCR and Fragment Analysis*

Genomic DNA samples of 50 Jersey crossbred cattle were amplified across 21 polymorphic microsatellite loci. Multiplex PCR [8,33] was used to reduce the time and cost of the process thereby increasing the efficacy of the test. The amplicons were then resolved with increased accuracy through fragment analysis using automated DNA analyzer.[14,15,17,19]

4.3 Validation of markers based on Genetic diversity parameters

4.3.1 Allelic richness

Among the 21 microsatellite loci analyzed, a total of 170 alleles were observed with a mean of 8.0952 ± 0.47 per locus. The number of observed alleles per locus had ranged from 4 (ILSTS11) to 12 (INRA23 and TGLA122), while the expected number of alleles ranged from 2.701(ETH3) to 6.485(CSSM66), with mean values of 8.095±0.47 and 4.388±0.24 respectively (Table2 and Figure2). The values observed in the study were in agreement with earlier reports in various Holstein Freisian populations[1,7] . The Indian and Chinese HF populations were found to have an average of 10.5 across 12 loci and 8.35 across 17 loci respectively; with the number of alleles per locus varying from 7 (ETH225 and ETH3) to 16 (INRA23) in the former and 6 (TGLA126) to 16 (TGLA122) in the latter. In 2002, Hansen *et al.*,[34] informed lower number of alleles in their study with the average per locus being 6.3 ± 0.64 for Canadienne, 6.3 ± 0.49 for Brown Swiss, 4.9 ± 0.47 for Jersey, and 6.1 ± 0.49 for Holstein cattle; while, Chikki *et al.*, 2004[35] found a mean of four alleles per locus across 12 loci among the 223 Jersey cattle.

Table 2. Genetic diversity parameters of microsatellite markers used for parentage verifications in Jersey crossbred cattle of Tamil Nadu.

Locus	No. of animals	No. of observed alleles (N_o)	No. of Expected alleles (N_e)	Observed Hetreozygosity (H_o)	Expected Hetreozygosity (H_e)	Polymorphism Information Content (PIC)
TGLA126	50	5	3.723	0.760	0.739	0.691
ILSTS006	50	8	4.985	0.800	0.808	0.773
INRA23	50	12	6.188	0.880	0.847	0.820
INRA63	45	9	4.232	0.689	0.772	0.731
HEL9	50	8	3.997	0.760	0.757	0.711
BM1824	49	5	2.959	0.653	0.669	0.606
ILSTS11	50	4	3.849	0.720	0.748	0.692
TGLA227	50	10	3.551	0.840	0.726	0.686
TGLA122	50	12	6.024	0.740	0.842	0.813
ETH10	50	8	5.214	0.940	0.816	0.783
CSSM66	50	10	6.485	0.920	0.854	0.830
CSRM60	14	5	3.769	0.929	0.762	0.689
BM2113	50	7	3.808	0.800	0.749	0.702
ETH3	50	7	2.701	0.780	0.636	0.592
SPS115	16	9	6.095	0.813	0.863	0.816
BM1818	50	8	4.744	0.860	0.797	0.764
ETH152	50	7	3.487	0.820	0.720	0.678
MM12	49	9	3.415	0.796	0.715	0.666
TGLA53	37	9	3.878	0.649	0.752	0.704
INRA5	48	9	3.759	0.750	0.742	0.705
ILSTS33	50	9	5.291	0.920	0.819	0.786
Mean± S.E.		8.095 ± 0.47	4.388 ± 0.24	0.801 ± 0.02	0.768 ± 0.02	0.726 ± 0.02

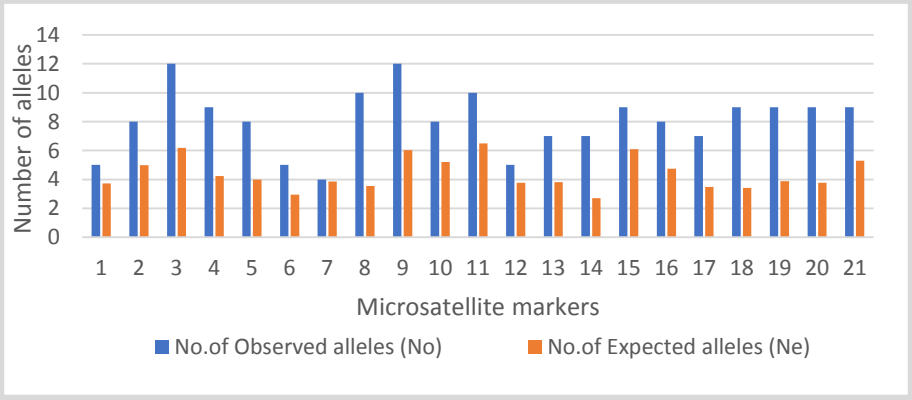

Fig2: *Observed and expected number of alleles in Jersey crossbred cattle of Tamil Nadu*

4.3.2 *Heterozygosity*

The observed heterozygosity values for the 21 loci ranged from 0.636(ETH3) to 0.863(SPS115) with a mean of 0.801±0.019 (Table2 and Figure 3) and similarly, the highest expected heterozgosity was observed at SPS115 (0.863) and lowest at ETH3 (0.636) with an overall mean of 0.768±0.013 in Jersey crossbred cattle population of Tamil Nadu used in this study. According to Beattie (1996)[36], the degree of variation at a marker locus influences the probability of detection to a rare dominant allele which segregates. Rehout *et al*., (2006)[6]had also reported high expected heterozygosity values in Czech Holstein Friesian population ranging from 0.607 (8 alleles, SPS115) to 0.835 (12 alleles, TGLA227), with an average value 0.746. Similarly, expected heterozygosity (He) values ranging from 0.581 (ETH225) to 0.873 (INRA23) and 0.554 (INRA063) to 0.828 (HEL9) were reported in Indian[1]and Chinese Holstein Friesian populations [7] respectively.

4.3.3 *Polymorphism Information Content (PIC)*

. Polymorphism information content (PIC)[27] of the microsatellite loci in this study ranged from 0.592(ETH3) to 0.830 (CSSM66) with an overall mean of 0.726 ± 0.02 (Table2and Figure 3) indicating that markers used in the study are highly polymorphic and suitable in assigning the parentage. Informativeness (polymorphism) represents by the probability that a given offspring of a parent carrying the rare allele allows deduction of the parental genotype at the marker locus[36]. Comparable PIC values were observed in Czech Holstein Friesian population ranging from 0.575 (SPS115, 8 alleles) to 0.816 (TGLA227, 12 alleles) with the average value of 0.713[6]. Indian HF population[1] had also revealed higher polymorphism of 0.6 in 11 loci among the 12 markers screened, while 8 out of 17 loci had PIC values higher than 0.7 in Chinese Holstein Friesian population [7].

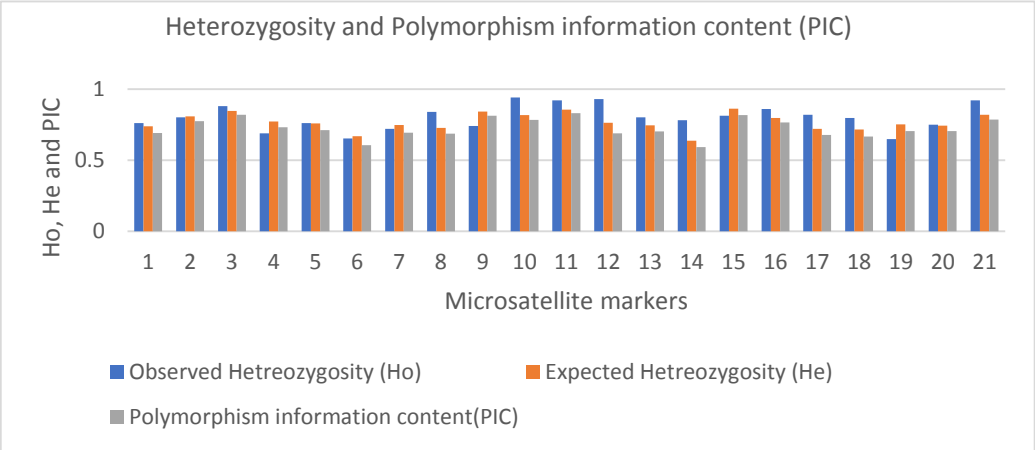

Fig 3 : Observed heterozygosity (Ho), expected heterozygosity (He) and Polymorphism information content (PIC) in Jersey crossbred cattle of Tamil Nadu

4.4 *Validation of markers based on Probability measures*

4.4.1 *Probability of Identity (PI)*

The PI for Jersey crossbred cattle ranged from 0.0396 at CSSM66 to 0.1744 at ETH3 with a mean of 0.0943 ± 0.008 (Table 3). The probability of identity is the probability that two randomly chosen individuals in a population have identical genotypes[7] and the actual observed PI for the number of codominant and dominant marker loci is required to be reasonably low (*i.e.,* 0.01–0.0001) [37]. The PI in this study reached 0.0000 with four loci combinations (Figure 4) which elucidates the efficiency of the markers in individual differentiation. Similarly, in a previous report, the probability of two random animals in Chinese Holstein breed having identical genotypes was estimated as 6.34×10^{-11} and 1.52×10^{-16} for the 10 and 17 set of loci, respectively[7].

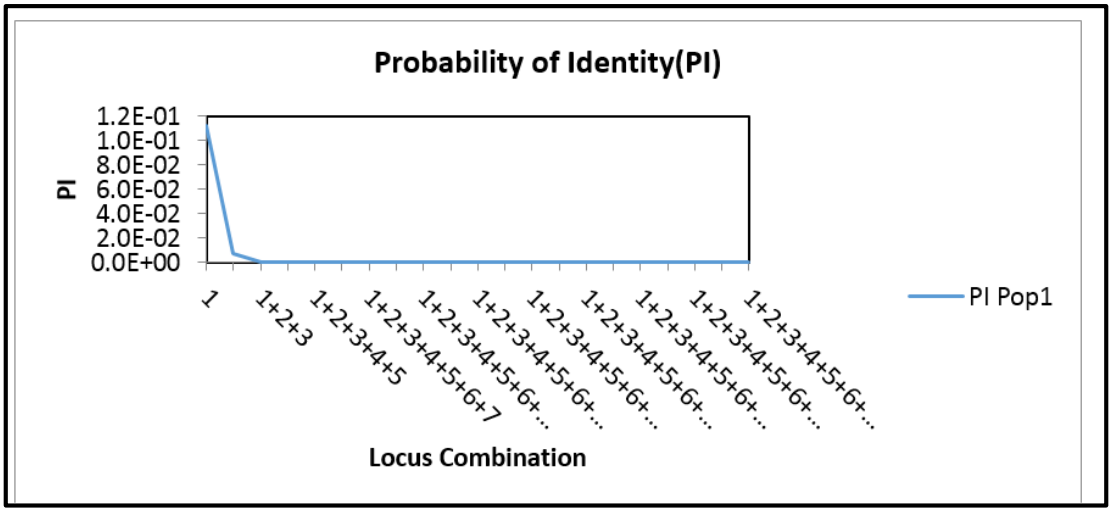

Fig 4 : Probability of identity (PI) of microsatellite markers used for parentage verifications in Jersey crossbred cattle

Table 3. Probability of identity (PI) and probability of exclusion (PE) of microsatellite markers used for parentage verification in Jersey crossbred cattle.

Locus	PI by locus	PI for increasing locus combinations	PE1 by locus - when the other parent is known	PE1 for increasing locus combinations	PE2 by locus - when genotype of one parent is missing	PE2 for increasing locus combinations	PE3 by locus - excluding a putative parent pair	PE3 for increasing locus combinations
TGLA126	**0.1123**	0.1123	0.5038	0.5038	0.3248	0.3248	0.6904	0.6904
ILSTS006	**0.0666**	0.0075	0.6141	0.8085	0.4367	0.6197	0.7991	0.9378
INRA23	**0.0447**	0.0003	0.6841	0.9395	0.5172	0.8164	0.8585	0.9912
INRA63	**0.0881**	0.0000	0.5614	0.9735	0.3827	0.8867	0.7531	0.9978
HEL9	**0.1012**	0.0000	0.5300	0.9875	0.3526	0.9266	0.7170	0.9994
BM1824	**0.1707**	0.0000	0.4067	0.9926	0.2440	0.9445	0.5825	0.9997
ILSTS11	**0.1155**	0.0000	0.4921	0.9962	0.3173	0.9621	0.6659	0.9999
TGLA227	**0.1113**	0.0000	0.5118	0.9982	0.3294	0.9746	0.7128	1.0000
TGLA122	**0.0483**	0.0000	0.6716	0.9994	0.5025	0.9874	0.8462	1.0000
ETH10	**0.0620**	0.0000	0.6276	0.9998	0.4518	0.9931	0.8102	1.0000
CSSM66	**0.0396**	0.0000	0.7002	0.9999	0.5348	0.9968	0.8726	1.0000
CSRM60	**0.1157**	0.0000	0.4970	1.0000	0.3221	0.9978	0.6777	1.0000
BM2113	**0.1042**	0.0000	0.5263	1.0000	0.3470	0.9986	0.7211	1.0000
ETH3	**0.1744**	0.0000	0.4078	1.0000	0.2308	0.9989	0.6019	1.0000
SPS115	**0.0468**	0.0000	0.6758	1.0000	0.5064	0.9995	0.8500	1.0000
BM1818	**0.0700**	0.0000	0.6043	1.0000	0.4249	0.9997	0.7940	1.0000
ETH152	**0.1177**	0.0000	0.4961	1.0000	0.3143	0.9998	0.6917	1.0000
MM12	**0.1265**	0.0000	0.4812	1.0000	0.3043	0.9999	0.6728	1.0000
TGLA53	**0.1043**	0.0000	0.5261	1.0000	0.3489	0.9999	0.7172	1.0000
INRA5	**0.0995**	0.0000	0.5354	1.0000	0.3517	0.9999	0.7373	1.0000
ILSTS33	**0.0602**	0.0000	0.6332	1.0000	0.4584	1.0000	0.8154	1.0000
Mean ± S.E.	0.0943 ± 0.008	0.0057 ± 0.005	0.5565 ± 0.02	0.9619 ± 0.02	0.3811 ± 0.02	0.9251 ± 0.04	0.7423 ± 0.02	0.9817 ± 0.01

4.4.2 Probability of Exclusion (PE)

All the three PE *viz.*, PE1 (when the other parent is known), PE2 (when genotype of one parent missing) and PE3 (excluding a putative pair) were highest at CSSM66 and lowest at BM1824 (Table 3). These loci have PIC values of highest (0.830) and lowest (0.592), thus indicating that the PIC is directly proportional to the ability of the markers in excluding the wrong parent. The probability of exclusion for increasing locus combinations reached 1.0000 with 8 markers for

PE3(Figure 5c), with 12 markers for PE1(Figure 5a), but with 21 markers for PE2(Figure 5b). The probability of exclusion (PE) is a measure of the ability of a certain panel of marker to identify genetic paternity, excluding all other candidates[7]. Exclusion probabilities in Czech Holstein cattle population were reported to correspond with their heterozygosity with the highest values in TGLA227, TGLA122 and INRA023[6]. Riojas-Valdes *et al*., (2009)[38] estimated the exclusion probabilities of 8 microsatellites as 0.9988 in Holstein, 0.9924 in Simmental, 0.9998 in Brown Swiss, 0.9999 in Beefmaster, 0.9930 in Brahman and 0.9990 in Brangus. Parentage testing using microsatellites in Yak revealed very high exclusion probabilities, only for the combined core set of 17 loci[8]. While, in river buffalo, a cumulative PE1 of nine marker loci was estimated to be 0.9999 while in case of absence of one of the parental genotypes, a minimum of 11 markers were required to achieve a cumulative PE2 of 0.999[39].

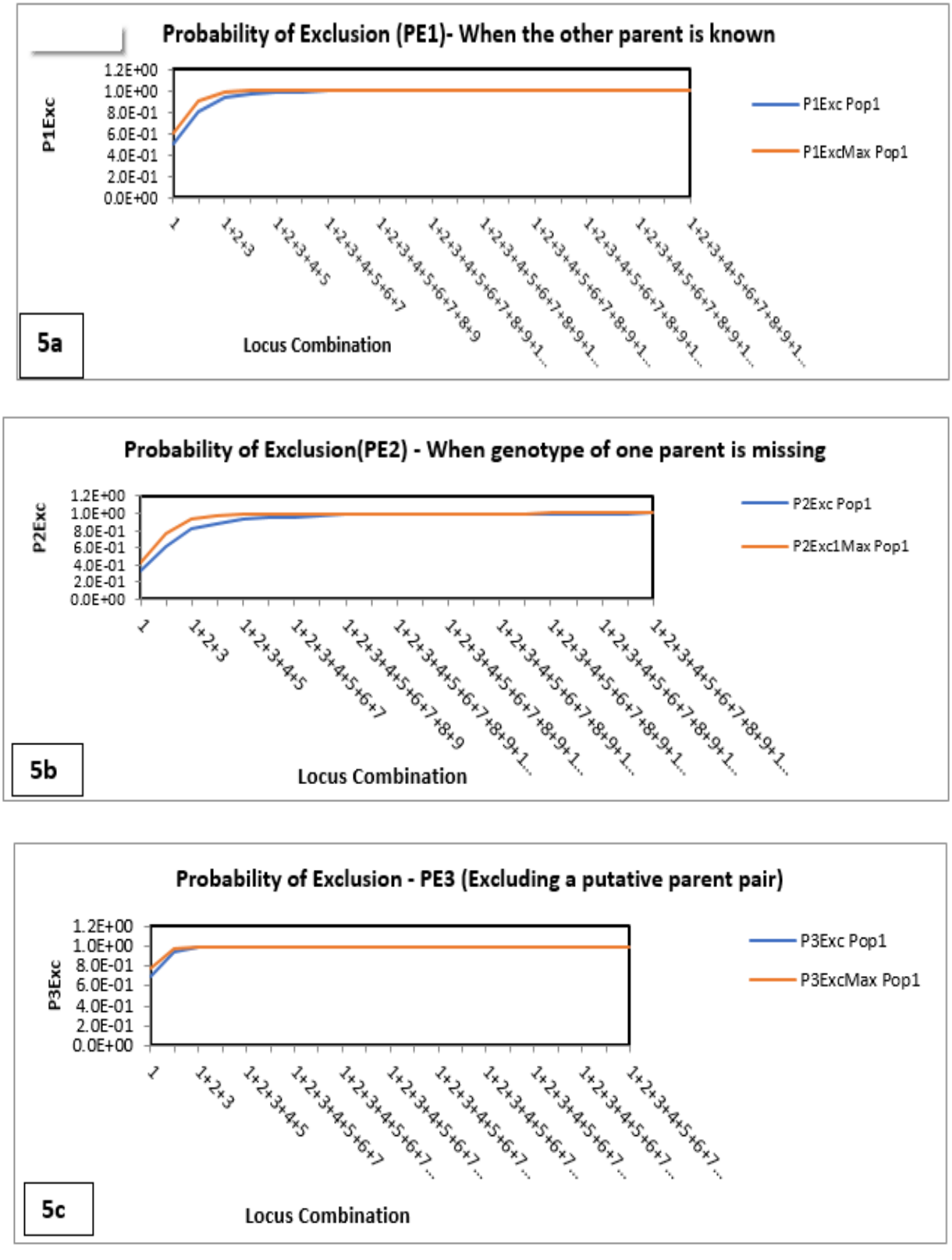

Fig 5 . Probability of Exclusion at (a)PE1, (b)PE2 and (c)PE3 levels of microsatellite markers used for parentage verifications in Jersey crossbred cattle

5. CONCLUSIONS

This study proves the efficiency of microsatellite markers in parentage tests due to its accuracy and cost effectiveness, inspite of fastgrowing SNP based parentage tests. The findings indicate that the markers selected in this study are highly informative for parentage testing of Jersey crossbred cattle of Tamil Nadu. Moreover, the study reveals sufficiently less number (8 or 12) of markers for effectively identifying the correct parentage when either a putative pair is excluded or only one parent is known.

6. AUTHORS' CONTRIBUTIONS

SMK Karthickeyan conceptualized the study and acquired the funds through GoI-NPBB-TNLDA project. The laboratory work, scoring and statistical analyses were done by senior author P Hepsibha. V Judia Harriet Sumathy was the research advisory. The original draft was written by Hepsibha P. Reviewing and editing of the article was done by SMK Karthickeyan and V Judia Harriet Sumathy. All authors read and approved the final manuscript.

7. ETHICAL APPROVAL

This research involves processing of cattle blood samples for genomic DNA isolation. Blood and semen samples were collected by veterinarians from farms and field animals and were sent to Department of Animal Genetics and Breeding, Madras Veterinary College (which is already authorized to treat and do research on animals) for parentage testing. The same samples were utilized for this study under the guidance of SMK Karthickeyan, Professor and Head, Department of Animal Genetics and Breeding, Madras Veterinary College and a co-author of this paper. The remaining research involves non-infectious materials.

8. ACKNOWLEDGEMENTS

We thank Dr. Sivaselvam S.N., Former Professor and Head, Department and Animal Genetics and Breeding, Madras Veterinary College, TANUVAS, Chennai-600 007, Tamil Nadu, India for his initial steps in getting permission from the institution and moral encouragement for making this research possible.

9. CONFLICT OF INTEREST

Conflict of interest declared none.

10. REFERENCES

1. Khade AS, Sawane MP, Pawar VD, Maurya BK, Nair RR. Validation of Microsatellite Markers for Parentage Verification in Indian HF Cattle. Int. J. Curr. Microbiol. App. Sci. 2019;8(3):1246-52.
2. Parentage Verification | National Dairy Development Board (nddb.coop) [Internet]. Available from: https://www.nddb.coop/calf/parentage-verification
3. Animal Husbandry Policy Note 2020-2021[Internet] by Animal Husbandry, Dairying and Fisheries Department, Government of Tamil Nadu (2020). Available from: https://cms.tn.gov.in/sites/default/files/documents/ah_e_pn_2020_21.pdf
4. Brenig B, Schütz E. Recent development of allele frequencies and exclusion probabilities of microsatellites used for parentage control in the German Holstein Friesian cattle population. BMC genetics. 2016 Dec;17(1):1-9.
5. García-Ruiz A, Wiggans GR, Ruiz-López FJ. Pedigree verification and parentage assignment using genomic information in the Mexican Holstein population. Journal of dairy science. 2019 Feb 1;102(2):1806-10.
6. Řehout V, Hradecká E, Čítek J. Evaluation of parentage testing in the Czech population of Holstein cattle. Czech Journal of Animal Science. 2006;51(12):503-9.
7. Zhang Y, Wang Y, Sun D, Yu Y, Zhang Y. Validation of 17 microsatellite markers for parentage verification and identity test in Chinese Holstein cattle. Asian-Australasian Journal of Animal Sciences. 2010 Feb 22;23(4):425-9.
8. Pei J, Bao P, Chu M, Liang C, Ding X, Wang H, Wu X, Guo X, Yan P. Evaluation of 17 microsatellite markers for parentage testing and individual identification of domestic yak (Bos grunniens). PeerJ. 2018 Nov 12;6:e5946.
9. Stormont C. Contribution of blood typing to dairy science progress. Journal of dairy science. 1967 Feb 1;50(2):253-60.
10. Heaton MP, Harhay GP, Bennett GL, Stone RT, Grosse WM, Casas E, Keele JW, Smith TP, Chitko-McKown CG, Laegreid WW. Selection and use of SNP markers for animal identification and paternity analysis in US beef cattle. Mammalian genome. 2002 May;13(5):272-81.
11. Estoup A, Jarne P, Cornuet JM. Homoplasy and mutation model at microsatellite loci and their consequences for population genetics analysis. Molecular ecology. 2002 Sep;11(9):1591-604.
12. Schlötterer C. Evolutionary dynamics of microsatellite DNA. Chromosoma. 2000 Sep;109(6):365-71.
13. FAO. Molecular genetic characterization of animal genetic resources. FAO Animal Production and Health Guidelines. No. 9. Rome; 2011.
14. Silva EC, McManus CM, Guimarães MP, Gouveia AM, Facó O, Pimentel DM, Caetano AR, Paiva SR. Validation of a microsatellite panel for parentage testing of locally adapted and commercial goats in Brazil. Genetics and Molecular Biology. 2014;37:54-60.
15. Sánchez-Velásquez JJ, Reyes-Flores LE, Yzásiga-Barrera C, Zelada-Mázmela E. Performance comparison of gel and capillary electrophoresis-based microsatellite genotyping strategies in a population research and kinship testing framework. BMC research notes. 2021 Dec;14(1):1-8.
16. Mitchelson KR, Cheng J. Capillary Electrophoresis of Nucleic Acids: Volume II: Practical Applications of Capillary Electrophoresis. Humana Press; 2001.
17. Mansor F, Zamri L, Hamzah SS. Electrophoretic techniques for the detection of human microsatellite

d19s884. The Malaysian journal of medical sciences: MJMS. 2015 Mar;22(2):18.
18. Shewale JG, Qi LI, Calandro LM. Principles, practice, and evolution of capillary electrophoresis as a tool for forensic DNA analysis. Forensic Science Review. 2012 Jul 1;24(2):79.
19. Wang ZF, Dai SP, Lian JY, Chen HF, Ye WH, Cao HL. Allele Size Miscalling due to the Pull-Up Effect Influencing Size Standard Calibration in Capillary Electrophoresis: A Case Study Using HEX Fluorescent Dye in Microsatellites. IntechOpen; 2018.
20. Sambrook J, Fritsch EF, Maniatis T. Molecular Cloning: A Laboratory Manual, 2nd cdn,('old Spring Harbor Laboratory. New York. 1989.
21. Wu H, de Gannes MK, Luchetti G, Pilsner JR. Rapid method for the isolation of mammalian sperm DNA. Biotechniques. 2015 Jun;58(6):293-300.
22. Madilindi MA, Banga CB, Bhebhe E, Sanarana YP, Nxumalo KS, Taela MG, Mapholi NO. Genetic differentiation and population structure of four Mozambican indigenous cattle populations. Livestock Research for Rural Development. 2019;31(4).
23. De Donato M, Brenneman RA, Stelly DM, Womack JE, Taylor JF. A methodological approach for the construction of a radiation hybrid map of bovine chromosome 5. Genetics and Molecular Biology. 2004;27:22-32.
24. Sharma R, Pandey AK, Singh Y, Prakash B. Evaluation of genetic variability in Ponwar cattle bymicrosatellite markers. Journal of Applied Animal Research. 2006 Sep 1;30(1):63-7.
25. Yeh FC. POPGENE (version 1.3. 1). Microsoft Window-Bases Freeware for Population Genetic Analysis. http://www. ualberta. ca/~ fyeh/. 1999.
26. Kalinowski ST, Taper ML, Marshall TC. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular ecology. 2007 Mar;16(5):1099-106.
27. Elston RC. Polymorphism information content. Encyclopedia of biostatistics. 2005 Jul 15;6.
28. Peakall R. PE smouse, 2006. GENALEX 6: genetic analysis in Excel. Population software for teaching and research. Mol. Ecol. Notes.;6:288-95.
29. Goud TS, Upadhyay RC, Kumar A, Karri S, Choudhary R, Ashraf S, Singh SV, Kumar OS, Kiranmai C. Novel extraction of high quality genomic DNA from frozen bovine blood samples by using detergent method. Open Veterinary Journal. 2018 Nov 12;8(4):415-22.
30. Hawken RJ, Cavanagh JA, Meadows JR, Khatkar MS, Husaini Y, Zenger KR, McClintock S, McClintock AE, Raadsma HW. Whole-genome amplification of DNA extracted from cattle semen samples. Journal of dairy science. 2006 Jun 1;89(6):2217-21.
31. Prośniak A, Gloc E, Berent J, Babol-Pokora K, Jacewicz R, Szram S. Estimating the efficiency of DNA isolation methods in semen, blood and saliva stains using the QuantiBlot system. Archiwum Medycyny Sadowej i Kryminologii. 2006 Jan 1;56(1):19-23.
32. Ghaheri M, Kahrizi D, Yari K, Babaie A, Suthar RS, Kazemi E. A comparative evaluation of four DNA extraction protocols from whole blood sample. Cellular and Molecular Biology. 2016 Mar 31;62(3):120-4.
33. Luikart G, Biju-Duval MP, Ertugrul O, Zagdsuren Y, Maudet C, Taberlet P. Power of 22 microsatellite markers in fluorescent multiplexes for parentage testing in goats (Capra hircus). Animal Genetics. 1999 Dec;30(6):431-8.
34. Hansen C, Shrestha JN, Parker RJ, Crow GH, McAlpine PJ, Derr JN. Genetic diversity among Canadienne, Brown Swiss, Holstein, and Jersey cattle of Canada based on 15 bovine microsatellite markers. Genome. 2002 Oct 1;45(5):897-904.
35. Chikhi L, Goossens B, Treanor A, Bruford MW. Population genetic structure of and inbreeding in an insular cattle breed, the Jersey, and its implications for genetic resource management. Heredity. 2004 May;92(5):396-401.
36. Beattie CW. Microsatellite heterozygosity: functional constraints on the development and use of comprehensive genomic maps for livestock. In Genomes of Plants and Animals 1996 (pp. 15-29). Springer, Boston, MA.
37. Waits LP, Luikart G, Taberlet P. Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. Molecular ecology. 2001 Jan;10(1):249-56.
38. Riojas-Valdes VM, Gomez-de-la-Fuente JC, Garza-Lozano JM, Gallardo-Blanco DC, De Tellitu-Schutz JN, Wong-Gonzalez A, Dávalos-Aranda G, Salinas-Meléndez JA. Exclusion probabilities of 8 DNA microsatellites in 6 cattle breeds from northeast Mexico. Journal of Animal and Veterinary Advances. 2009;8(1):62-6.
39. Kathiravan P, Kataria RS, Mishra BP. Power of exclusion of 19 microsatellite markers for parentage testing in river buffalo (Bubalus bubalis). Molecular biology reports. 2012 Aug;39(8):8217-23