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Microsatellite marker-based genetic diversity in Mareecha and Barela breeds of dromedary camel from Pakistan

*T. Hussain, F. Hussain, A. Wajid, M. Ellahi Babar, M. M. Musthafa and F. M. M. T. Marikar**

Abstract

The genetic diversity of Pakistani dromedary camels is poorly documented. The present study evaluated the genetic variations of two well-known Pakistani camel breeds, Mareecha and Barela, that are well-adapted to the Cholistan desert climate. Camel can serve as a beneficial participant in the food supply chain by providing milk, meat and other food products for the livelihood of pastoral peoples. To explore a new world of resources, greater attention to needed to create standard procedures to genetically characterize, classify and identify camel breeds in the country. For this purpose, 66 unrelated animals of the Mareecha (*n*=35) and Barela (*n*=31) breeds were genotyped using a set of 12 labelled microsatellite loci. DNA fragment sizes were determined in an ABI 3130 Genetic Analyzer. All microsatellite markers were successfully amplified and exhibited a polymorphic nature, with an average Polymorphic Information Content (PIC) of 0.72 and 0.70 in Mareecha and Barela, respectively. A total of 107 alleles with an average of 8.91 alleles per locus were identified by these markers in both breeds. CMS15 was highly polymorphic with 13 alleles, while VOLP-032 was the lowest with two loci. The F_{it} and F_{is} values were low but high population differentiation (17%) was observed in both breeds, due to the migrations of pastoral people to different remote areas during climate fluctuations.

Key words: *Camel breeds; Genetic Diversity; Microsatellite; Pakistan*

Tanveer HUSSAIN#, DVM, PhD, Full Professor, Department of Molecular Biology, Virtual University of Pakistan; Fiaz HUSSAIN#, DVM, PhD, School Education Department, Dera Ghazi Khan, Punjab; Abdul WAJID, DVM, PhD, Department of Biotechnology, Virtual University of Pakistan, Pakistan; Masroor ELLAHI BABAR, DVM, PhD, The University of Agriculture, Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan; Muneeb M. MUSTHAFA, DVM, PhD, Department of Biosystems Technology, Faculty of Technology, South Eastern University of Sri Lanka, University Park, Oluvil, #32360, Sri Lanka; Faiz M. M. T. MARIKAR*, DVM, PhD, (Corresponding author, e-mail: faiz.marikar@fulbrightmail.org), General Sir John Kotelawala Defense University, Ratmalana, Sri Lanka; Faculty of Health Sciences, The Open University of Sri Lanka, Nawala, Sri Lanka
#Authors contributed equally

Introduction

Camel is a domestic animal indigenous to many regions in the world, including Pakistan. Camel domestication began in the Arabian Peninsula around 3000 B.C. (Compagnoni and Tosi, 1978), and this animal is an important member of the livestock sector, often called the "ship of the desert" (Đuričić et al., 2020a,b). Dromedaries are multipurpose animals utilized as substantial providers of conveyance, meat and milk, as well as hair (Raziq and Younas, 2006; Đuričić, 2019). They are also popular in sports, as camel racing is a paramount industry in the Middle East and many other countries (Ahamed et al., 2010). Desert vegetation and thorny plants form the basis of the camel's diet (Mikesell, 1955; Mehta and Sahanp, 2007; Đuričić, 2017). The Food and Agriculture Organization has estimated that the global camel population in 2014 was about 26.7 million (Joint, 2004), of which about 90% are dromedaries. In Pakistan, the camel population is estimated at more than 1 million camels (Hussain et al., 2013), most of which are one-humped though some are Bactrian camels, found typically in the northern areas. In four ecological zones, i.e. sandy desert, coastal mangroves, mountainous tracts, and irrigated plains, camels are predominantly the riverine and mountainous types (Ahamed et al., 2010; Ishani and Baloch, 2010).

Though protein polymorphism has been widely used for genetic characterization, it has shown very little genetic variation in camels (Guerouli and Acharbane, 2005). On the other hand, microsatellites have shown great promise for genetic characterization globally (Gautam et al., 2004; Mehta and Sahani, 2007; Mahmoud et al., 2012; Musthafa, 2015). An initial set of microsatellites has been used in studies on camelids from United Arab Emirates, Germany, Australia, Kenya and Ethiopia (Han et al., 2000), and also on alpacas and llamas (Lang et al., 1996; Obreque et al., 1998; Penedo et al., 1999). This set was comprised of sixteen primers with the highest polymorphism. However, assessing diversity in camel genetics globally is not limited only to these markers, and a range of different microsatellite markers in camels has been used according to Musthafa, (2015).

Genetic diversity studies on camels are limited, though in recent years, several studies have been conducted using microsatellite markers (Mburu et al., 2003; Hashim et al., 2015; Almathen et al., 2016), mitochondrial DNA (mtDNA) sequences (Babar et al., 2015; Almathen et al., 2016) and candidate gene sequencing (Pauciullo et al., 2013; Shuiep et al., 2013; Almathen et al., 2016). Bahbahani et al. (2019) conducted a study on full genome sequencing of camels from the Arabian Peninsula and the genotyping-by-sequencing of Sudanese camels to assess their genome diversities, relationships, and candidate signatures of positive selection. Unfortunately, there is a lack of camel genetic diversity studies on Pakistani camels. Therefore, the objective of this study was to assess the genetic diversity of Barela and Mareecha camel breeds from Pakistan.

Materials and methods

This study was designed to investigate the genetic variation in the two most prominent camel breeds, *i.e.* Mareecha and Barela, that inhabit and are adapted to harsh desert climates, such as the Cholistan Desert in Punjab. A total of 66 samples (Mareecha *n*=35, Barela *n*=31), with known phenotypic characteristics were randomly collected for blood sampling. Upon obtaining approval from the institutional ethics committee, blood was drawn from the jugular vein using a sterile syringe. Blood was transferred to EDTA coated vacutainer tubes and

immediately put on ice and stored at -20°C in the lab. Extraction of whole genomic DNA was done from whole blood (5 mL) with minor modification of the Sambrook procedure (Sambrook et al., 1989).

A panel of 12 microsatellite labelled markers (CMS13, CMS15, CMS17, CVRL02, LCA66, VOLP03, VOLP08, VOLP10, VOLP32, VOLP67, YWLL38, YWLL44) recommended by the Food and Agriculture Organization and International Society for Animal Genetics (FAO/ISAG) dispersed across the whole genome and displaying polymorphism were selected and tested for genetic analysis in both selected camel breeds (Table 1). Each forward primer of all twelve-microsatellite marker was 5′ labelled with three different (6-FAM, TET, HEX) fluorescence tags in order to perform fragment length analysis of the PCR product with ABI PRISM 3130 XL Genetic Analyzer (Applied Biosystems, USA). Three different Multiplex combinations were used to amplify these markers. PCR amplification was done on a Thermo cycler (Bio-Rad, USA) using a reaction mixture of 25 μL containing 50 ng/μL genomic template, 5U Taq polymerase enzyme (Thermo Scientific USA), 2.5 mM each dNTPs, 2.5 μL 10× buffer and 2.5 mM of $MgCl_2$. PCR condition was used as an initial denaturation of 5 minutes at 95 °C followed by 35 cycles; each cycle consisted of three phases: denaturation for 45 s at 94 °C, next annealing for 45 s at 52 °C and an extension for 45 s at 72

Table 1. FAO and ISAG recommended camel microsatellites markers used to investigate the genetic diversity in Mareecha and Barela camel breeds of Pakistan

Name	Gene Accession No	Primers 5′-3′	5′ Labeled	Size Range	References
CMS-13	AF329158.1	**F:** TAGCCTGACTCTATCCATTTCTC **R:** ATTATTTGGAATTCAACTGTAAGG	HEX	238-265	(Bareta et al., 2013)
CMS15	AF329151.1	**F:** AAATACTTAAAGGTTCCCAGA **R:** TTGTAAACTAAAGCCAGAAAG	6-FAM	121–144	(Bareta et al., 2013)
CMS17	AF329147.1	**F:** TATAAAGGATCACTGCCTTC **R:** AAAATGAACCTCCATAAAGTTAG	HEX	135-167	(Bareta et al., 2013)
CVRL02	AF217602.1	**F:** TGTCACAAATGGCAAGAT **R:** AGTGTACGTAGCAGCATTATTT	TET	205–215	(Penedo et al., 1999)
LCA66	AF091125.1	**F:** GTGCAGCGTCCAAATAGTCA **R:** CCAGCATCGTCCAGTATTCA	6-FAM	212-262	(Mehta and Sahanp, 2007)
VOLP03	AF305228.1	**F:** AGACGGTTGGGAAGGTGGTA **R:** CGACAGCAAGGCACAGGA	TET	129-206	(Mburu et al., 2003)
VOLP08	AF305230.1	**F:** CCATTCACCCCATCTCTC **R:** TCGCCAGTGACCTTATTTAGA	HEX	142-180	(Mburu et al., 2003)
VOLP10	AF305231.1	**F:** CTTTCTCCTTTCCTCCCTACT **R:** CGTCCACTTCCTTCATTTC	6-FAM	242–268	(Mburu et al., 2003)
VOLP32	AF305234.1	**F:** GTGATCGGAATGGCTTGAAA **R:** CAGCGAGCACCTGAAAGAA	6-FAM	192-262	(Mburu et al., 2003)
VOLP67	AF305237.1	**F:** TTAGAGGGTCTATCCAGTTTC **R:** TGGACCTAAAAGAGTGGAG	TET	142-203	(Mburu et al., 2003)
YWLL38	GU723275.1	**F:** GGCCTAAATCCTACTAGAC **R:** CCTCTCACTCTTGTTCTCCTC	6-FAM	174-192	(Guerouli and Acharbane 2005)
YWLL44	GU723276.1	**F:** CTCAACAATGCTAGACCTTGG **R:** GAGAACACAGGCTGGTGAATA	HEX	86-120	(Guerouli and Acharbane 2005)

°C were selected in PCR machine. Final extension was carried out at 72 °C for 10 minutes.

Bioinformatics Analysis

Microsatellite marker-based genetic diversity in the Mareecha and Barela breeds was analysed to determine allelic frequencies, mean number of alleles per locus (NA), observed (Ho) and expected (He) heterozygosities using POPGENE version 1.31 (Yeh and Young, 1999). For the calculation of F statistics: F_{it} (total inbreeding), F_{st} (estimate of population differentiation) and F_{is} (within population in breeding estimate), GENEPOP version 4.0 software was used (Raymond and Rousset, 1995). The level of significance was determined using FSTAT software. Chi-square tests for Hardy-Weinberg equilibrium for each locus in two populations and across populations were performed using POPGENE version 1.31 (Yeh and Young, 1999).

Results and discussion

All markers were successfully amplified and showed a polymorphic nature. A total of 107 different alleles across the 12 microsatellite loci were identified, with an average of 8.91 alleles per locus in both breeds. The number of polymorphic alleles per locus ranged from 3 to 13 (mean 8.50) and 2 to 10 (mean 6.33) in the Mareecha and Barela breeds, respectively. The number of alleles is most dependent on sample size (Mahmoud et al., 2012). All other genetic parameters are given in Table 2.

Genetic diversity was determined in llama camelids where 506 alleles (Barreta et al., 2013) were identified using 43 STR markers. In Moroccan camel pop-

Table 2. Descriptive statics of all twelve microsatellites markers in both the Mareecha and Barela breeds

Breeds	Mareecha					Barela				
Locus	Na	Ne	Ho	He	PIC	Na	Ne	Ho	He	PIC
YWLL38	9.00	6.38	0.88	0.86	0.83	7.00	3.23	1.00	0.72	0.66
LCA66	9.00	4.11	0.60	0.77	0.72	5.00	3.90	0.91	0.78	0.70
CVRL02	8.00	4.79	0.88	0.81	0.77	6.00	3.41	1.00	0.74	0.66
CMS17	6.00	3.54	0.96	0.73	0.68	4.00	3.14	0.91	0.71	0.63
CMS15	13.00	5.53	1.00	0.84	0.80	10.00	6.05	1.00	0.87	0.82
CMS13	10.00	3.94	0.68	0.76	0.72	7.00	5.38	0.73	0.85	0.79
VOLP032	3.00	1.73	0.32	0.43	0.59	2.00	1.94	0.45	0.51	0.50
VOLP67	8.00	3.48	0.96	0.73	0.67	5.00	3.32	1.00	0.73	0.65
YWLL44	9.00	4.43	0.80	0.79	0.75	7.00	6.72	0.82	0.89	0.83
VOLP08	9.00	4.03	0.96	0.77	0.72	8.00	5.38	0.91	0.85	0.79
VOLP03	9.00	5.87	0.96	0.85	0.81	8.00	6.21	0.55	0.88	0.82
VOLP10	10.00	5.81	0.76	0.84	0.81	7.00	5.50	0.73	0.86	0.79
Mean	8.50	4.47	0.81	0.76	0.72	6.33	4.51	0.83	0.78	0.70
St. Dev	2.24	1.30	0.20	0.11		2.10	1.53	0.19	0.11	

Na= Number of alleles; Ne= effective number of alleles; Ho= observed heterozygosity; He=expected heterozygosity; PIC= Polymorphic Information Contents.

ulations, 79 alleles were identified using seven molecular markers (Piro et al., 2011), while 139 dissimilar alleles and an average number of 10.7 alleles over 13 loci (Schulz et al., 2010), whereas in the same year, an allele range of 5-23 was reported for Australian camel by using total 28 markers, identifying 185 different alleles (Spencer et al., 2010). A total of 51 alleles were identified using 16 STRs marker in the Kachachi camel population (Parikh et al., 2012). Numerical and molecular diversity data on South African camels was reported by Karak et al. (2017). They used 12 microsatellites markers for the genetic diversity of camels. In Bikaneri camels, 2-7 alleles were identified using 16 microsatellites markers, and 50 individuals of Kachachi camel were genotyped using 16 STR markers, with a reported allele range of 2-6 (Mehta and Sahanp, 2007). In Indian dromedary camels, a total of 252 alleles were found by using 23 microsatellites markers across four populations with a mean number of alleles per locus of 8.04, 7.30, 6.39, and 7.43 for Bikaneri, Jaisalmeri, Kutchi, and Mewari breeds, respectively (Vijh et al., 2007). The observed number of alleles per locus in Mareecha (8.50) and Barela (6.33) breeds is significantly higher than a previous study on Canarian camels (2.22; Schulz et al., 2010), and Australian camels (5.23; Spencer et al., 2010). The overall mean observed heterozygosity in Pakistani Mareecha and Barela camels was 0.8194. This shows that the observed heterozygosity of Pakistani dromedaries is higher than for other dromedary camels as the mean observed heterozygosity in Majheem camels is 0.665 (Mahmoud et al., 2012), Tunisian camels 0.455 (Ahmed et al., 2010). However, the heterozygosity value was 0.552 in Arabian camels and the values were 0.580, 0.570, 0.560 and 0.600 in Indian camels Bikaneri, Jaisalmeri, Kutchi and Mewari, respectively (Vijh et al., 2007). The expected heterozygosity value of Majheem camel was 0.652, analogous with African and Arabian camels but lower than Sudanese camels (Mukasa-Mugerwa, 1981; Walsh, 2001). The homozygosity of Pakistani Mareecha and Barela was 0.1806 and 0.1667, respectively.

The Polymorphic Information Content (PIC) is a parameter indicator of the degree of informativeness of microsatellite markers. The PIC ranged from 0.50 (VOLP-32) to 0.82 (YWLL44) in Barela camels and from 0.59 (VOLP-32) to 0.83 (YWLL38) in Mareecha. The average PIC values were 0.72 and 0.70 in Mareecha and Barela breeds, respectively, while in the Indian Kachchhi camel breed, it ranged from 0.277 to 0.765 (Mehta et al., 2007). The average PIC value was 0.511 in racing camels (Spencer et al., 2010) and 0.427 in Iran bactrian camels (Afraz et al., 1998), which was lower than for Pakistani dromedary camels. The Shannon's index ranged from 0.3960 to 1.5087 (Parikh et al., 2012).

Fixation indices

Calculations of the F-statistic in the tested population were F_{is} -0.1013, F_{it} -0.0826 and F_{st} 0.0170. All these estimates significantly differed from zero (P<0.05). In a Bolivian llama population, the overall F_{is} value was estimated at 0.08636 for 12 regional groups. The mean estimates of F statistics in Indian dromedaries were F_{it} =0.227, F_{is} =0.157 and F_{st}=0.082, while in Saudi camels, the mean estimates were F_{is} = -0.043, F_{it} = -0.025 and F_{st} = 0.018 (Mahmoud et al., 2012). The F_{is} value for Canarian camels was 0.04. In Canarian dromedaries, the F_{st} statistic ranged from 0.095–0.116 (Schulz et al., 2010). In the population of Tunisian dromedaries, the mean estimates of F statistics were F_{it}=0.27, F_{is}=0.19 and F_{st}=0.09 (Ahmed et al., 2010). The summary of F statistics is given in Table 3.

Gene flow

Nm values are responsible for gene flow, and the higher the gene flow, the lower the differentiation between

Table 3. Summary of F-statistics and gene flow for 12 loci in selected populations Mareecha and Barela camel breeds in Pakistan

Locus	F_{is}	F_{it}	F_{st}	N_m
YWLL38	-0.23	-0.20	0.02	10.90
LCA66	-0.01	0.00	0.01	47.60
CVRL02	-0.26	-0.23	0.02	13.20
CMS17	-0.34	-0.32	0.01	17.24
CMS15	-0.21	-0.19	0.01	20.35
CMS13	0.10	0.13	0.03	7.06
VOLP032	0.15	0.17	0.03	9.67
VOLP67	-0.39	-0.38	0.01	39.32
YWLL44	0.00	0.03	0.03	8.29
VOLP08	-0.19	-0.18	0.01	31.50
VOLP03	0.10	0.12	0.02	11.66
VOLP10	0.10	0.10	0.01	27.27
Mean	-0.10	-0.08	0.02	14.44

populations. Nm and F_{st} values are in inverse proportions. In this study, LCA66 markers contained the highest Nm value (47.6) and lowest F_{st} (0.0052) value.

The similarities between two camel breeds may be due to large scale gene flow from one population to another. This may also be due to inbreeding within the population estimate, which represents the non-random union of gametes and deviation from Hardy-Weinberg Equilibrium. It is known that migration, natural processes of mutation, non-random mating, genetic drift, and both artificial and natural selection are factors that cause deviations from HWE. In reported camel populations, the mean Nm value was 14.4, and were highest in Indian dromedary camel, indicating a higher observed gene flow (Nm=39.7) between the Sofr and Shual populations, and lowest (Nm=14.5) between the Magaheem and Maghateer populations (Vijh et al., 2007). Limited gene flow was observed between Jaisalmeri and Mewari camel breeds (Nm=1.29) and high between Jaisalmeri and Kutchi breeds (Nm=15.58). The Nm values among Tunisian camel breeds were 1.65 between Kebili and Medenine, 2.06 between Kebili and Tataouine and 6.65 between Medenine and Totaouine populations (Mukasa-Mugerwa, 1981; Ahmed et al., 2010). The observed Nm values was 9.061 between South African and Sudanese camels, 1.157 between South African camels and alpacas, and 1.388 between Sudanese camel population and alpacas (Mahmoud et al., 2012).

Conclusions

This is the first report of microsatellite markers for the Mareecha and Barela camel breeds in Pakistan. Further studies and collaborative research approaches are required to better understand and utilize this unique animal as a potential animal of the future.

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Genetska raznolikost Mareecha i Barela pasmina jednogrbih deva iz Pakistana na temelju mikrosatelitskih markera

Dr. sc. Tanveer HUSSAIN, dr. med. vet., redoviti profesor, Zavod za molekularnu biologiju, Univerzitet Virtual u Pakistanu, Pakistan; dr. sc. Fiaz HUSSAIN, dr. med. vet., Odjel školskog obrazovanja, Dera Ghazi Khan, Punjab; dr. sc. Abdul WAJID, dr. med. vet., Zavod za biotehnogiju, Univerzitet Virtual u Pakistanu, Paksitan; dr. sc. Masroor ELLAHI BABAR, dr. med. vet., Poljoprivredni Univerzitet, Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan; dr. sc. Muneeb M. MUSTHAFA, dr. med. vet., Zavod za biosistemsku tehnologiju, Tehnološki fakultet, Jugoistočni Univerzitet u Šri Lanki, Univerzitetski park, Oluvil, #32360, Šri Lanka; dr. sc. Faiz M. M. T. MARIKAR, Univerzitet General Sir John Kotelawala Defense, Ratmalana, Šri Lanka; Fakultet zdravstvenih znanosti, Open Univerzitet u Šri Lanki, Nawala, Šri Lanka

Genetska raznolikost pakistanskih jednogrbih deva slabo je dokumentirana. Ova studija procijenila je genetske varijacije dviju dobro poznatih pasmina pakistanskih deva - mareecha i barela - koje su vrlo dobro prilagođene klimi pustinje Cholistan. Deva može poslužiti kao koristan sudionik u lancu opskrbe hranom, osiguravajući mlijeko, meso i druge prehrambene proizvode za život pastira. Da bi se istražio novi svijet resursa, potrebno je više pozornosti za obavljanje standardnih postupaka za genetsku karakterizaciju, razvrstavanje i identifikaciju pasmina deva u zemlji. U tu svrhu, ukupno 66 životinja koje nisu u srodstvu pasmine mareecha (*n*=35) i barela (*n*=31) genotipizirane su uporabom 12 označenih mikrosatelitskih lokusa. Veličine fragmenata DNK određene su u ABI 3130 genetskom analizatoru. Svi mikrosatelitski markeri uspješno su pojačani i pokazali su polimorfnu narav s prosječnim sadržajem polimorfnih informacija (PIC) od 0,72 i 0,70 za mareecha, odnosno barela pasminu. Ukupno 107 alela s prosječno 8,91 alelom po lokusu u obje pasmine identificirano je tim markerima. CMS15 bio je vrlo polimorfan s 13 alela dok je VOLP-032 bio najniži s 2 lokusa. F_{it} i F_{is} vrijednosti bile su niske, ali je uočena velika diferencijacija populacije (17 %) za obje pasmine koje su posljedica migracije pastira u različite udaljene krajeve tijekom promjena klime.

Ključne riječi: *pasmine deva, genetska raznolikost, mikrosatelit, Pakistan*