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Short Communication

Mitochondrial Phylogeny and Population Structure of Pakistani Dromedary Camel (*Camelus dromedarius*)

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Abstract

Dromedary camels (*Camelus dromedarius*) are one of the most important livestock species mainly used for milk and meat production in semi-arid and hot-desert expanses of the Arabian-Peninsula, Africa, and Southwest Asia. This study investigated the genetic diversity and population structure within and between eight dromedary camel breeds (n = 210) inhabiting Balochistan province, Pakistan, by mitochondrial cytochrome b (*Cyt b*). Sequences (1140 bp) analysis showed a total of 18 variable sites resulting in 16 haplotypes. The average haplotype and nucleotide diversities were Hd = 0.484 ± 0.051 and π = 0.00272 respectively. Phylogenetic analysis showed different clusters for camelids. The neutrality tests did not support the population demographic expansion for these camel breeds. Based on these results, we suggest that an imperative genetic management and breeding strategies are required for the effective conservation of this species. © 2021 Friends Science Publishers

Keywords: *Cyt b*; Haplotypes; Phylogeny; Dromedary camels; Balochistan

Introduction

The genus *Camelus* is an interesting species inhabiting cold and hot deserts and as well as high and low altitude worldwide. The family Camelidae is divided into two groups, New-World (Lamini) and Old-World (Camelini) camels. Within Camelini, two domesticated species are described including the single-humped dromedary or Arabian camel, (*Camelus dromedaries*) found in Middle-East, Horn of Africa and Southeast of Asia (Bahbahani *et al.* 2019) and the two-humped Bactrian camel (*Camelus bacterianus*) found in Central and Eastern-Asia, as well as the critically endangered two-humped wild camel (*Camelus ferus*) distributed in remote areas of the Gobi and Taklamakan deserts in Mongolia and Northwest China (Yadamsuren *et al*. 2019; Fitak *et al.* 2020).

The world population of the camel is estimated at around 35 million with the majority (90%) are the dromedaries (FAO 2019). In Pakistan, the population of the camel is estimated to be around 1.0 million distributed in arid, semi-arid, and desert areas of the country with 20 different recognized breeds. They have been providing rich organic milk, meat, hides, wool, dancing/racing animal, and transport facility provider. Camels are an important part of the country's economy and have a significant role in the socio-economic uplift of the local community. The hardy nature of camel with wonderful physiological characteristics has made it the best-suited animal for draft purposes in the climatically harsh and inaccessible regions (Chen *et al.* 2019).

Dromedaries are one of the major pastoral domestic species in Pakistan along with few herds of Bactrian camels found in Northern areas. Balochistan shares a total of 41% of dromedary camels in Pakistan, followed by Punjab (22%), Sindh (30%) and Khyber Pakhtunkhawa (7%) respectively. Balochistan is situated in the southwestern part of Pakistan in a desert belt between 25ºN to 32ºN latitude and 60ºE to 72ºE longitude. Balochistan contains mostly arid and semi-arid areas, deserts and mountainous regions. Camels are an important animal in the province's livestock herds and play a vital role in the economy of rural pastoral communities. Overall, the camel is still a neglected species in Pakistan and has not given proper attention by scientists and researchers. Comprehensive information on the

population structure and diversity found in dromedaries from Balochistan province is still missing. To fill this gap and to understand the phylogenetic relationship between populations, we investigated the genetic diversity among eight camel breeds using mitochondrial *Cyt b* gene sequences. With this study we aim to provide first genetic data for genetic-based breed management in dromedaries of Balochistan province.

Materials and Methods

In total, we sampled 210 animals from eight dromedary camel breeds in geographically different districts of Balochistan province (Fig. 1), including the Brahvi, Kharani, Kachhi, Mukrani, Roadbari, Pishin, Lassi and Kohi well adapted to hot, arid and cold, dry environments of Balochistan. The blood samples were collected in EDTA-containing vacutainer-tubes and were carried on ice-box to Animal Genomic Laboratories, Virtual University of Pakistan. The DNA was isolated from the blood using revised phenol/chloroform procedure previously described by Wajid *et al*. (2014). The extracted DNA samples were quantified using the Nanodrop spectrophotometer (Thermo-scientific, U.S.A.) and stored at -20ºC until further use. The complete *Cyt b* gene was amplified and sequenced using three-overlapping primers previously reported by Babar *et al*. (2015). PCR amplification was carried out in a final volume of 30 μL mixture contained genomic DNA (20 ng) 2 μL, forward and reverse primers (10 pmol) 1.5 μL, dNTPs (2.5 m*M*) 2 μL, reaction-buffer (10X) 3.5 μL, $MgCl_2$ (1.5 m*M*) 3.5 μL, Taq DNA-polymerase (5Unit) 0.5 μL (Thermo-scientific) and H_2O 17 μL. The amplification was carried out in BioRad Thermos-cycler using initial denaturation of 95ºC for 5 min followed by 33 cycles of denaturation at 95ºC for the 30s, annealing at 60ºC for 30s and extension at 72ºC for 1 min and then with a final extension at 72ºC for 7 min. The purified *Cyt b* gene PCR products were sequenced by ABI3130 automated-sequencer (ABI, Inc., Foster City, C.A.). A Maximum Likelihood method phylogenetic tree was constructed to investigate the evolutionary relationship of the studied camel breeds with other dromedary, and Bactrian camel breeds using MEGAv6 software. DnaSP v. 5 program was used for conducting neutrality tests, *i.e*., Tajima's D and Fu and Li's F to detect a deviation from neutrality, and to calculate within species diversity parameters, *e.g.*, number of haplotypes, nucleotide and haplotype diversities. The complete *Cyt b* haplotypes (n = 16) obtained in this study were submitted to GenBank and are available under accession number MT578032 to MT578047.

Results

The mtDNA sequence analysis of 1140 bp in 210 animals showed a total of 18 variable sites resulting in 16 different haplotypes that demonstrated a moderate mtDNA genetic diversity in the Pakistani camel populations. The detail of the number of variations in each breed, haplotype, haplotype diversities, and nucleotide diversities are shown in Table 1. Among the studied camel breeds, the average haplotype and nucleotide diversities were Hd= 0.484 ± 0.051 and π = 0.00272 respectively. Highest number of haplotypes (n = 3) was detected in Pishin and Kharani dromedaries while only a single haplotype was identified in Kachhi and Rodbari breeds, respectively. The haplotype diversity ranged from Hd = 0.500 ± 0.048 in the three populations Brahvi, Kohi, and Lassi to Hd = 0.690 ± 0.023 in Pishin and Kharani (Table 1). Nucleotide diversity ranged from π = 0.00044 (Lassi) to π = 0.00242 (Kharani). The haplotypes identified in camel populations under study were unique to each population, except Markrani and Rodbari shared one haplotype; this is a likely crossbreeding as geographically nearest neighbors.

The Rodbari camel breeds showed highest genetic relatedness (0.1%) with other two Makrani and Lassi camel breeds. Among all studied breeds, Kachhi was divergent camel exhibited 1.3% genetic distance from Makrani and Kharani camel breeds. A Maximum Likelihood method phylogenetic tree was constructed to investigate the evolutionary history of the studied camel breeds with other dromedary and Bactrian camels from Arabian Peninsula, Iran, Ethopia, Kenya, Russia, China and Mongolia. The result clearly exhibited two distinct clades, dromedary and Bactrian. The Bactrian camels further divided into two different lineages, the domestic and wild animals (Fig. 2). Two classical tests were employed to detect deviation from neutrality. We obtained positive values of Tajima's D and Fu and Li's F tests in all studied camel breeds, indicative of balancing selection or a population subdivision event, but the result was statistically significant (P < 0.05) only in three camel breeds.

Discussion

In this study, we investigated the genetic diversity and phylogenetic relationship among 210 dromedaries belonging to eight camel breeds reared in Baluchistan province using complete mitochondrial *Cyto b* amplification. Retrieving this genetic information is a first indispensable step to facilitate the breed conservation program in an effective and reminiscent way. Camel ecotype is not well defined in Pakistan and very little information is available on genetic-studies in camels found all over the country. Hence, the molecular studies on genetic diversity in this specie can facilitate the development of national camel-breeding program to establish an effective strategy for the conservation of these important genetic resources.

Cyt b is a highly conserved region of mitochondrial DNA (mtDNA) genome used for studying species classification and detection of phylogenetic relations among diverse mammalian species.

Table 1: The genetic diversity analysis of studied camel populations

Species	Samples	Detected variations	H	Haplotype diversity (Hd)	Nucleotide diversity (Pi)	K	Tajima's D	*P* value	Fu and Li's F
Pishin	30	2	3	0.690 ± 0.023	0.00081	0.91954	1.68264	P > 0.10	1.21673 P > 0.10
Brahvi	25	3	2	0.500 ± 0.048	0.00132	1.50000	2.20530	P < 0.05*	1.52520 P < 0.05*
Kachhi	25	0	1	0.000	0.00000	0.00000	0.00000	0.00	0.00000
Kharani	30	6	3	0.690 ± 0.023	0.00242	2.75900	2.34410	P < 0.05*	1.80694 P < 0.05*
Kohi	25	2	2	0.500 ± 0.048	0.00088	1.00000	1.92637	P > 0.10	1.30700 P > 0.10
Lassi	25	1	2	0.500 ± 0.048	0.00044	0.50000	1.47274	P > 0.10	0.97500 P > 0.10
Makrani	25	4	2	0.513 ± 0.037	0.00180	2.05300	2.53549	P < 0.01*	1.73014 P < 0.05*
Rodbari	25	0	1	0.000	0.00000	0.00000	0.00000	0.00	0.00000

H: Haplotype
K: the average number of nucleotide differences

Fig. 1: Geographical locations of the samples used in this study

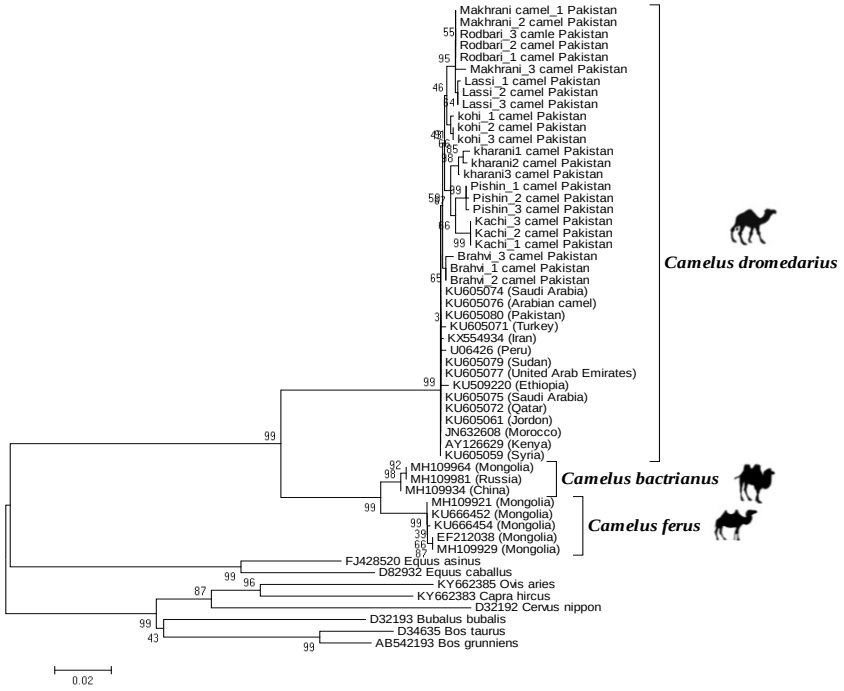

Fig. 2: Maximum-Likelihood method phylogenetic tree inferred from *Cyto b* gene sequences

The partial and complete *Cyt b* has been used for detection of genetic conservation and phylogenetic relationship among Bactrian camel (Ming *et al.* 2016), dromedaries (Babar *et al.* 2015; Legesse *et al.* 2018) and other livestock species (Hussain *et al.* 2018). In this study, complete *Cyto b* gene has been used for comparing genetic

diversity between eight camel breeds rearing in different geographical regions of Balochistan. The breeds showed low differences in their genetic diversity. The haplotypes identified were unique to each breed except the two camel breeds except Markrani and Rodbari shared one haplotype. The uniqueness of the haplotypes identified in the studied camel populations generally suggests the haplotypes specific to a geographic region and low level of crossbreeding.

Two classical tests were employed to detect deviation from neutrality. The positive D values indicate an excess of intermediate frequency alleles and may suggest balancing selection or population contraction. Neutrality tests were not performed on Kachhi and Rodbari camel breeds, because these species had no genetic variation in the *Cyt b* gene. The neutrality tests showed no population demographic expansion for the eight studied camel populations. Phylogenetic analysis was performed based on the complete *Cyt b* gene and showed different clusters for camelids. The phylogeny of the *Cyt b* gene was consistent with known phylogeny for the ruminant classification based on other genes (Iqbal *et al.* 2020).

Conclusion

This study provides initial information on the genetic diversity of camel populations reared in Balochistan province. The studied camel populations exhibited low levels of haplotype and nucleotide diversities, which may be due to the small effective population size or many years inbreeding that may further diminish the genetic diversity in these camel populations. Indeed, the camels are well adaptable domestic animals to the harsh ecosystems where these animals are essential for the economy and food security of local communities. We suggest based on these results, an imperative genetic management and breeding strategies are required for effective conservation of this species.

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Author Contributions

AF, AW and MTM designed and perceived the experiment, AF, MEB and AW execute the experiment, AF and TH collected the samples and AF and TH helped in data analysis, AW wrote the manuscript.

Conflicts of Interest

All authors declare no conflicts of interest.

Data Availability

Data presented in this study are available on fair request to the corresponding author.

Ethics Approval

The experiments were carried out in accordance with the guidelines issued by the Ethical Committee of University of Balochistan, Quetta, Pakistan.

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