GENETIC CHARACTERIZATION OF LASI AND MAKRANI CAMEL BREEDS OF LASBELA, BALOCHISTAN BY USING MICROSATELLITE MARKERS

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Abstract

Camel is a heat resistant animal reared in desert and hot areas for meat, milk production and for draught purpose. In the past camels have been used as a major source for commercial transportation. Beside the other districts of Balochistan of Pakistan, Lasbela district has a reasonable population of camels consisting 2.4% of the total livestock population. The prominent camel breeds are Lasi and Makrani with an emerging new hybrid (Dati) of both the known breeds. Microsatellite markers have widely been used to characterize the genetic diversification among the camel breeds. Current study performed on 40 samples from the 3 breeds of the district. Genomic DNA was extracted, amplified by using specific primers for the microsatellite markers under study. The sequencing results revealed >30 dimer GT repeats with a break of a dimer (TT) and in 5 samples the GT repeats were found continuously in heterozygous condition. Results of the current study suggest that microsatellite markers can have better use for genetic characterization among different breeds.

Keywords: Camel, Hybrid, Lasi, Makrani, Markers, Microsatellite

INTRODUCTION

Camel is a heat resistant animal and mostly reared in desert areas for milk, meat production and draught purposes. Species of camel (Camelidae) C. dromedarius, C. bacterianus, L.glama and V. pacos have been reported to be domesticated in different parts of the world (1, 2).

Lasbela is the Southeastern district of Balochistan covering 15153 square kilometers of land area with a vast costal area starting from Haux bay Karachi to the Ormara town of the Makran Division. Agriculture and Livestock are the main sources of income for the local population of the district. Beside the other small and large ruminants, camels accounting 2.4% of the total livestock population in the district are also reared in the range lands of the area. Camel milk and meat is used for food purposes, moreover, ice cream from Lasi camel breed has been processed and evaluated for physiochemical and sensory
characteristics (3). According to the 2006 livestock census the camel population in Balochistan was reported to be 41% of the total camel population of Pakistan and Lasbela is one of the districts with highest number of camels in Balochistan. In Balochistan all the camel breeds belong to the single humped Arabian Dromedary camel (C. dromedarius) that constitute about 94% of the total camel population of the world. Dromedary camels are among the last major domesticated livestock species, domesticated about the third millennium BCE (4-6). Almathen et al. in their study conducted by using the microsatellite markers and mitochondrial ancient DNA suggest the domestication of the dromedary camel possibly originated from Arabian Peninsula (7).

According to Livestock and Dairy Development Department, Government of Balochistan, there are different camel breeds like Kharani, Khurasani, Kohi, Reki (Raigi), Jathansal, Lasi and Makrani etc., reared in different districts in vast desert areas of Kharan, Chagai, Nushki, Lasbela, Makran and the mountainous areas of Kalat, Makran, Kohlu, Dera Bugti, Lorali, and Qilla Saifullah. However, the breeds raised in Balochistan are non-descriptive both phenotypically and genotypically. Very little literature is available regarding the genotypic characterization of the breeds.

Microsatellite/ STR (Short Tandem Repeat) markers have extensively been investigated in genetic studies. These markers have been reported in eukaryotes and found throughout the genome in both exonic and intronic regions and having a mutation rate of 10^3 -10^6/ cell generation with a degree of 10 orders higher than point mutations (8-10).

The objective of the current study was to investigate genetic variations using already published microsatellite markers, among the Lasi, Makrani and Dati camel breeds raised in the rangeland desert areas of Lasbela district, Balochistan.

**MATERIALS AND METHODS**

Current study was performed on 45 unrelated animals (August 2020 to March 2021) 15 from each Lasi, Makrani and Dati (hybrid of Lasi and Makrani breeds) camel breeds of Lasbela. Venous blood samples (5-8 ml) were collected from different localities (Bela-Uthal and Winder) of Lasbela District and dispensed into 15 ml falcon tubes containing Ethylenediaminetetraacetic acid (EDTA) as an anticoagulant agent. The samples were stored at -35°C before DNA extraction. Genomic DNA was extracted from whole blood samples by an inorganic method already published (11). DNA samples were amplified by utilizing the already published (12) primers that were synthesized from Thermo Fisher Scientific, Custom DNA Oligos Synthesis Services (Table 1).

<table>
<thead>
<tr>
<th>Locus</th>
<th>Primers</th>
<th>Primer Sequence</th>
<th>Length</th>
<th>Annealing Temperature</th>
<th>Product Size</th>
</tr>
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<tbody>
<tr>
<td>YWLL02</td>
<td>Primer Forward</td>
<td>GTGCACCTCAGATACCTTCACA</td>
<td>21</td>
<td>57.8</td>
<td>298-318</td>
</tr>
<tr>
<td></td>
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<td>53.4</td>
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<tr>
<td>YWLL38</td>
<td>Primer Forward</td>
<td>GCCCTAATTCCTACTAGAC</td>
<td>19</td>
<td>52.7</td>
<td>236-246</td>
</tr>
<tr>
<td></td>
<td>Primer Reverse</td>
<td>CCTCTCACCTGTTCTCCTC</td>
<td>21</td>
<td>57.8</td>
<td></td>
</tr>
<tr>
<td>VOLP10</td>
<td>Primer Forward</td>
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<td>21</td>
<td>55.9</td>
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</tr>
<tr>
<td></td>
<td>Primer Reverse</td>
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<td>52.7</td>
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<tr>
<td>VOLP77</td>
<td>Primer Forward</td>
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<tr>
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<tr>
<td></td>
<td>Primer Reverse</td>
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<td>LCA90</td>
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<td>55.4</td>
<td>237-248</td>
</tr>
<tr>
<td></td>
<td>Primer Reverse</td>
<td>CCAAGTAGATTTCTCTTATGCG</td>
<td>22</td>
<td>54.4</td>
<td></td>
</tr>
</tbody>
</table>

DNA samples were amplified by PCR using a 30 µl reaction volume including 2.5 µl 5x PCR buffer, 2.5µl MgCl2, 2µl dNTPs mix (1mM), 0.8 µl Taq DNA polymerase. 0.4 µl of forward and reverse primers each and the sample volume brought up to 30 µl by adding PCR graded (nuclease free) water. All the samples were amplified using 6 pairs of microsatellite markers with same sample volume by T 100 Thermal Cycler (BIO-RAD). Thermal cycling conditions were set as 96°C hold temperature for five minutes followed by...
denaturation at 95°C for 45 seconds; annealing temperature was set as per the recommended temperature given in (Table I) for 45 seconds and extension at 72°C for 1 minute for 30 cycles followed by a final extension at 72°C for 5 minutes.

Amplified products were sequenced (Tsingke Biotechnology, Co., Ltd., Beijing, China) by using automated 3100 ABI prism DNA genetic analyzer big dye terminator chemistry (12) for investigating any possible variation among and within the breeds under study.

RESULTS

Adult unrelated animals were selected for sampling from the selected breeds i.e., Lasi, Makrani and Dati breeds (Fig. 1). Samples having primers of VOLP10 microsatellite marker (Fig. 2) with the product size of 236-246 base pairs (Table I) whereas the other five pairs of markers showed no results.

Fig. 1. (a) Lasi, (b) Makrani and (c) Dati camel breeds reared in Lasbela District

Fig. 2 (a & b). Bands of amplified microsatellite marker, VOLP10
Amplified DNA samples were sequenced for variation analysis. Sequencing results revealed > 30 Guanine and Thymine (GT) repeats. In 35 samples a TT dimer is found after 9 GT repeats (Fig. 3).

Whereas, in 5 samples including 3 from Lasi camel breed and 2 from Dati breed the GT repeats were found continuous in heterozygous condition (Fig. 4).

DISCUSSION

Microsatellite markers have extensively been used to investigate genetic variations among livestock genetic resources (13-16). Microsatellite markers are reported to have existed in almost all live forms ranging from prokaryotes to eukaryotes (9, 10). The existence of molecular markers was reported first by Hamada et al., (17) followed by Delseny et al., (18), Tautz and Renz (19). Studies suggest that for analyzing genetic variation, molecular markers are useful tools that support the genetic and phenotypic variations among breeds/species (20). In the current study we identified 30 GT repeats with a break of a dimer TT (Fig. 4) whereas in 5 samples (3 of Lasi and 2 of Dati breeds) at the same position of TT dimer the GT repeats were found continuous in heterozygous condition. Study carried out by Sadder et al., (16) utilizing multiple loci reported dimer repeats of AC/CA/TG/GT ranging from 5-149 with 83 occurrences including many other dimer, trimer and tetramer repeats and suggested that microsatellite markers can be used as potential candidate loci for analyzing the genetic variations among camel breeds. Mehta and Sahani (21) in their study reported polymorphic microsatellite loci by using a diverse range of microsatellite markers including VOLP 10 and suggest that the frequency of diverse alleles associated with multiple markers loci is an indicator of genetic diversity in camels. Study carried out by Cherifi et al., (22) reported that the VOLP32 was the most informative locus for the genetic diversity among Egyptian camels. Similarly, Mahmoud et al., (23) in their study suggest microsatellite markers can effectively be used to characterize genetically diverse camel breeds.

CONCLUSION

In current study the VOLP10 microsatellite marker sequencing results showed diverse genetic variations in the camel breeds of Lasbela District, Balochistan. Heterozygous variation observed in Lasi and Dati breeds suggest close relationship between the two breeds but these results do not confirm the variations related to the specific breeds as the sample size is small and there exist no controlled breeding strategies to conserve the pure breeds. The results further indicate and suggest that VOLP10 along with other microsatellite markers can be proved helpful to characterize genetically the diverse camel population of Balochistan.

Conflict of Interest

The authors declare no conflict of interest.
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References:


