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A study about phylogenetic relationships of Bactriancamels' population in Iran using mitochondrial HVR-1

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ABSTRACT

Camels are not only important in improving rural families' economy, but also are valuable genetic resources. Maintaining genetic diversity of Bactrian camels in Iranis essential for breeding programs and increasing production because of Low population.The first step is determining the genetic diversity. Among genetic markers,mitochondrial genome sequencing is one of the best and most popular methods for classification of close populations and species, investigating the possibility of various species deviation from a common ancestor, studyingphylogenetic relationship of everycreature with other species,races, and finding methods for saving genetic resources. The goal of this study was determining the sequence of HVE-1region of a population of IranianBactrian camels. To this, blood samples were taken from 20 camels randomly. After DNA extraction, HVR-1 region was amplified by specific primers using PCR method. Amplified fragments were purified and then sequenced.Afterobtaining similar sequences of mitochondrial genome from other extent races in world gene bank, phylogenetic tree was drawn. Phylogenetic results showed that IranianBactrian camels are in same groupof Middle East which is probably because of very close genetic relationship of Iranian camels to Asian races.

Keywords: mitochondrial genome, highly variable regions, phylogeny, Bactrian camel

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INTRODUCTION

Native breeds are national capitalof each country. Protecting and reproducing these breedsare really important and valuable. Reduction in population of IranianBactriancamelhas caused some concernsabout the preservation of this unique race. Identifying genetic and functional characteristics of the remaining population of the endangered animalsis really necessary for management of this endangered livestock including phenotype properties, yield properties, quantitative traitslocating (QTL), importance of nurturing and finally saving and increasingremainingpopulation [1]. One way to identify this race is using mitochondrial genome. Mitochondrion is a cytoplasmicorganellewhich is found in most of body cells. It produces energy for cells and has specific cyclic DNA independent from nucleus DNA and encodes 37 genes in animal races including 13 respiratory chain encoding genes, 22 tRNAencoding genes and two RNA encoding genes.

The length of mitochondrion is about 16bp in birds [2]. Among genetic markers, sequencing of mitochondrion genome is one of the best and most popular methods for classification of close populations and species, investigating the possibility of various species deviation from a common ancestor, studying phylogenetic relationship of every creature with other species, races, and finding methods for saving genetic resources. Today, sequencing various mitochondrion genome parts isoneof the most applicable procedures for studying biodiversity and evaluating species and races because of mutation 10 times more than nuclear DNA and unprotected region which don't encode any protein and amplificationby specific primers[3].Siadatian et al. (2007) studied D-loop region of mitochondrial DNA

of 16 fish samples from two CaspianMullet fish species toidentify nucleotide sequence of them and determined the phylogenetic relation of them by molecular and morphological studies. Results showed that each species was located in separate branch with some differences [4].

Gohaetal. (2006) by using b-cytochrome markersand s r RNA 16 from mitochondrion genome identified phylogenetic relationships betweendeer and cow and could obtain theKinship amount [5].

Considering the increasing importance of camel, it is essential to have sufficient studies on nourishment, reproduction, breeding, diseases and other related categories of this animal to have a healthy food reservoir and also higher export income.Therefore, our goal of this study was determining phylogenetic relationship, deviationstudy and calculating genetic distance of Bactrian population from other camel races.

MATERIALS AND METHODS

The study was carried out in Islamic Azad University-SavehBranch (2013-2014). Blood samples were taken from jugular vein of 20 Ardabil Bactrian camels by using EDTA anticoagulant containingvenojects. DNA extraction was done usingDIAtomDNA Prep 100 (Diatom Company). Since DNApurity was really important, spectrophotometry method was used and electrophoresis on agars gel (0.8) was used for its quality.

Primer premier program was used to plan primers for amplification ofa part of control region from mitochondrial genome (a length of 1200bp). Forward primers were attached in 15444-15464 distance whereas reverse primers were attached in 16624-16643 distance from mitochondrion genome. Sequence of primers was asfollows:

Forward: 5'-AAAACggCAATAgCCCTTgAg-3'
Reverse: 5’- gCCCCCgTAAAATTgCTgTT-3'

These worldwide sequences were used to produce target points for sequence primers after producing chain reaction products. Polymerase chain reaction was done by using hot starts (Qiagen Inc., Germany) in DNA mixed Taq (Final volume 20 micro liter including 50 nanogramof genome and 6 picomole of each primer).

Thermalprogramincludedan initialdenaturationat94$^{\circ C}$ for3 min, 30cycles ofthermaldenaturationat95$^{\circ C}$ for30 s, junction attemperature55$^{\circ C}$for 45seconds, amplification at72$^{\circ C}$ for90seconds, and final amplification at 72° C for10min. To ensure from the size of considered fragment on gel, samples and 0.1% agarose(Merck, Germany) were electrophoresed in 1.5% concentration.

After electrophoresis and ensuring that non-specific bands and breaks don’t exist, samples were purified to delete small DNA pieces and probable primer dimer. To this,NucleoSpin Extract II Purification kit made by Macherey-Nagel MN Company (Germany) was used which is made for DNA extraction from TAE/TBE agarose gel and direct PCR products.

Consensus sequence was determined using SEQMAN program and this sequence was recorded on NCBI database. To draw phylogenetic graph, Neighbor- joining procedure of MEGA4 program was used. Forphylogenetic comparisons of HVR1 zone three phylogenies were drawn:one with samples sequences, one for Bactrian camel with other races in world for haplotype group determination and one for sequence of Bactrian camel with other extent races in group A.

RESULTS AND DISCUSSION

NanoDropspectrophotometer results showed that DNA extracted from Bactrian camel blood had good quality and quantity. One of the most important goals of this study was determining an index sequence for Bactrian camel and determining consensus sequence is one of the popular methods for recording and identification of different races.To this, synchronized samples for determining consensus sequence were obtained using BioEdit software and consensus sequence (850 bp length as index sequence for this race) asfollows:

Figure1. Consensus sequence of Bactrian camel

Evolutionary relations of organisms are shown by phylogenetictree. Since evolution occurs over long periods and cannot be sawn directly, biologists have to rebuild phylogenies by inference from evolutionary relations between present organisms. Nowadays, molecular data including protein and DNA strings are used for pedigree determination and making phylogenetic trees.

In this study, HVR1 zone was used for studying phylogenetic relationships. At first, HVR1 consensus sequence was compared with extent sequences in NCBIdatabase (under Blast process). Among this process eleven sequence of HVR1 zone of camel genome from various countries -which had coverage with study's regions- were received and aligned with sequence of this study under clusterprocedure of MEGN 5 program genetic distances between sequence of studied species were calculated by CLC work bench 5 program (figure 2).

		1	2	3	4	5	6	7	8	9	10	11	12
IRAN	1		0.00	0.00	0.00	0.00	0.01	0.03	0.03	0.03	0.06	0.06	0.06
GQ201526	2	100.00		0.00	0.00	0.00	0.01	0.03	0.03	0.03	0.06	0.06	0.06
EF212037	3	100.00	100.00		0.00	0.00	0.01	0.03	0.03	0.03	0.06	0.06	0.06
AP003423	4	100.00	100.00	100.00		0.00	0.01	0.03	0.03	0.03	0.06	0.06	0.06
EF507799	5	100.00	100.00	100.00	100.00		0.01	0.03	0.03	0.03	0.06	0.06	0.06
EF507800	6	98.89	98.89	98.89	98.89	98.89		0.02	0.02	0.02	0.06	0.06	0.06
JN632608	7	96.68	96.68	96.68	96.68	96.68	97.78		0.00	0.00	0.04	0.04	0.04
EU159113	8	96.68	96.68	96.68	96.68	96.68	97.78	100.00		0.00	0.04	0.04	0.04
AB753152	9	96.68	96.68	96.68	96.68	96.68	97.78	100.00	100.00		0.04	0.04	0.04
EU285663	10	94.10	94.10	94.10	94.10	94.10	94.10	96.31	96.31	96.31		0.00	0.00
AJ566364	11	94.10	94.10	94.10	94.10	94.10	94.10	96.31	96.31	96.31	100.00		0.00
AP003426	12	94.10	94.10	94.10	94.10	94.10	94.10	96.31	96.31	96.31	100.00	100.00	

Figure2. Genetic distancesmatrix of Bactrian camel (HVR1 region of mitochondrion genome) with other NCBI extent races (above the diameter genetic distance amount andbelow the diameter genetic similarity percentage)

The matrix of genetic distances and phylogeny graph (figure 3) of HVR1 region sequence of Bactrian camel and extent camel races in NCBI shows that this camel is closest to Bactrian camels of Middle East which is probably due to very close genetic relationships of Iranian Bactrian camel with Asian races.

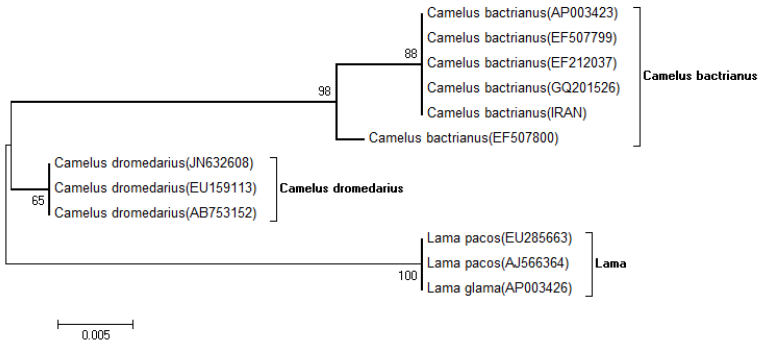

Figure3. Phylogenetic graph according to general sequence of Bactrian camel and some camel races in world gene bank plus their access code

These results are in agreement with studies of Valizadeh et al. which showed that humped cattle (Sistani) and without humps cattle (Sarabi) were really close even they were in two separate haplotype groups. This may show close origin of these races [6].

Also, our results in agreement with studies ofLee et al. (2006) which studied D-loop zone of Chinese cows' mitochondrion. Phylogenic analysis of these sequences in both humped cattle and without humps cattle led to dividing them in two main groups, northern and southern cows [7].

In this study also, sequence of HVR1 zone of studied samples was compared with eleven recorded sequences (NCBI data base) from different countries and results showed that Iranian Bactrian camels have less genetic difference from Middle EasternBactrian camels which is probably because of close genetic relationships of them.

CONCLUSION

Comparing HVR1 region of Bactrian camels with single humped camels shows high difference between two races. Considering that Bactrian camels of Iran are endangered and embryo transferring of this animal is being executed with 50 camels, the risk of inbreeding and following dangers exist. Mitochondrial DNA is one of the most importantmarkersfor studying genetic relationships and phylogenetic, therefore, using D-loop marker and b-cytochrome in researches (i. e. embryo transferring) is proposed for saving biodiversity.

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