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Genetic Variation of Indian Camel (*Camelus dromedarius*) Breeds Using Mitochondrion COI Gene Analysis

Pramathadhip Paul[1], Payal Pal[1], and Ena Ray Banerjee[1]

[1]Immunology and Regenerative Medicine Research Laboratory, Department of Zoology, University of Calcutta, Kolkata, India.

*Corresponding author: Banerjee ER, Department of Zoology, University of Calcutta, Kolkata, India, Tel: 9163739975; E-mail: enaraybanerjee[at]caluniv.in

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Abstract

Mitochondrial cytochrome c oxidase subunit I (COI) gene is the most conservative protein-coding gene in the mitochondrial genome of different animals. Universal primers are used to amplify 710 bp fragment of the COI gene. In India, there are four different breeds of camel present in different region - Bikaneri, Kuchchhi, Jaisalmeri, and Mewari. The aim of this article is to phylogenetic study based on the COI sequence analysis of these diverse animals. There are two advantages to focus on COI gene a) Universal primer; b) COI shows greater range of phylogenetic signal. Compared to other genes which codes protein, third position nucleotides of the COI gene show highly base substitutions, and therefore suitable for highest rate of molecular evolution, greater than 12S or 16S rDNA analysis. We are also reporting likelihood values, functional divergence in amino acid changes and polymorphism among four camel breeds in India. We have identified some unique mutations in the individual breed in India and therefore divergent from the main species.

Keywords: ***COI gene; Phylogenetic; Camelus dromedaries.***

1. Abbreviations

NRCC: National Research Centre on Camel; **ICAR:** Indian Council of Agricultural Research; **COI:** Cytochrome C Oxidase Subunit I; **RFLP:** Restriction fragment length polymorphism; **RAPD:** Random amplified polymorphic DNA; **AFLP:** Amplified fragment length polymorphism; **SNP** Single Nucleotide Polymorphism

2. Introduction

Among the mammals, Camel has a highly adaptive ability to a specific ecosystem (the desert or high altitude). It has a remarkable adaptation to harsh conditions like high temperature tolerance due to low basal metabolism and also water

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conserving ability. Physiology of the animal possess low respiration, perspiration and variation of the body temperature of up to 6°C. Camel is distinguished by its variant diets herbaceous plants, date stones, shrubs, and cacti. However, the number of the Camel is become declining in India. Camels are members of Camelidae family, suborder- Tylopoda, order- Artiodactyla, class- Mammalian [1]. Camels were cultivated around 300 BC. Single humped dromedary camels (*Camelus dromedaries*) found in western region of the India. About 0.63 million dromedarian camels are available in India, which is 3.3% of the total population of the world. The National Research Centre on Camel (NRCC), India has reported that a total of 19.31 million camels present in world wide. Among this 15.13 million camel present in Africa, 4.17 million camel present in Asia. Male dromedaries are around 10% heavier than female, and are 400-600 kgs. Their shoulder height is also 10 cm taller than female. In addition to this, male dromedaries have an inflatable soft palate to attract females. Dromedarian camels are categorized by four breeds- Bikaneri, Kuchchhi, Jaisalmeri and Mewari. Breeding region of the Mewari Camel is in Arawali, Mountains of the Rajasthan, India. These animals are lighter in weight and shorter than Bikeneri. They are well adapted for travel and carry goods in the hill area. Body colour of the animals is light brown to dark brown. Among these, some animals are white.

Bikaneri camels are massive height and have good body stature. They have stop- a "hollow above eyes." It is the major breeds in India. This breed is derived from the city Bikaner, established by Rao Bika in the 15th century. Colour of the body is brown to black. They have a slightly domed shaped head. The forehead of the animal is well-marked depression (Stop) above the eyes. Some animals of this breed have luxuriant hair growth in eyebrows, eyelids and ears. They are called Jheepra. Neck of this breed is thick, reasonably erect.

The Kachchi breeds are found in the Gujrat region. They do not have a stop. These animals are linked with social status. The major breeding tract encompasses the Kachchh and Bansakantha district in Gujrat. The body colour of this breed is brown to dark brown. They do not have hair on eyelids and ears. Their head is medium size without a distinct stop. The body of the animals is short and stouter. Their hard and heavy foot pads are adapted for the humid climate and marshy land of Kachchh. Some animal shows teeth due to lower droopy lip in their face.

The breeding tract of the Jaisalmeri camels are found in Jaisalmer, Barmer and Jodhpur district of the Rajasthan. This breed is quite tall with long legs. They have small head and mouth with narrow muzzle. Their head is not doom shaped and is without depression (stop). Hairs are absent on their eyebrows, eyelids and ears. Body colour of the animal is light brown and has very thin skin with short hairs.

There are several genetic studies was done on the camelidae family in Dubai, Australia, Germany, Kenya, Ethiopia based on the microsatellite loci. In contrast, alpacas and llama were found new microsatellite [2-4].

Camel is used in various purpose; transport (carting, riding), leisure (tourism, camel dance, festivals), agricultural work (weeding, water spreading, weeding), production of wool, milk, milk products such as ice cream, milk powder, coffee, kulfi, skin, manure, meat and most importantly development of nanobody etc. "Pushkar" is the most popular and biggest Camel fair in Rajasthan, India. Camels are well decorated by ornaments; the whole body is designed by hair cutting. Therefore, Pushkar become a tourist spot. Presently milk products are trendy from camel milk. NRCC group are

preparing ice cream, kulfi and coffee for sale. Other products are also top-rated like paper from camel dung, dhuries (rugs) from wool. In Rajasthan region, camel carts are used for local transportation. Even the skin of the Camel is also an essential raw material to prepare leather bags, belts, shoes and jackets, which are directly sold in leather factories. State dairies also start business of popularize camel milk which is more nutritional than cow's milk. It is reported that foreigners describe as the romantic image of a camel ride through the Thar Desert. They described as the most unforgettable experience when camel ride in the desert.

Indian Council of Agricultural Research (ICAR), NRCC initiates to develop camel health and its products. They start a direct self-help group for camel farmers who are dealing with milk products. They form a co-operative society, 'Sarahi,' for trading camel milk. Even they initiate helping groups in different milk collection centers. Scientists from NRCC interact to direct farmers for clean milk production, breed improvement methods, various disease control agenda, and also improvement in the marketing of camel milk.

For camel health improvement policy, various research projects are going on in NRCC, Rajasthan, India. Projects are – Genetic improvement of the milk production potential of Indian dromedary (2007-2012), structural analysis of milk protein gene of Camel (2011-2014), epidemiology of infectious diseases of Camel, management of GI parasites in Camel (2007-2012), improving the efficiency of artificial insemination in Camel (2008-2012), enhancing nutrient utilization and reducing methane emission (2009-2012), evaluation of feed pellets containing different protein levels in camel calves (2011-2012), improvement of feed resources and nutrient utilization in raising animal production (2003-2012), bioprospecting of genes and allele mining for heat and cold stress tolerance in Indian dromedaries (2009-2012), development of new camelid anti-snake venome (2007 to long term), development of single-domain antibodies for diagnosis/therapy (2007 to long term) (Annual report, 2011-12; NRCC).

Dromedary camels are the most common species among the camel family and are widespread in southwest Asia. Based on their morphological characters, they are well distinguished. For example: - erythrocytes of the family Camelidae are elliptical shapes. Camel erythrocytes are incredibly resistant to osmotic lysis [5]. Morphological characters of the Camelidae family have different body hair colour, forehead is distinguished depression (stop), their different height, etc.

Molecular marker studies among the family of the Camelidae are the new techniques for biodiversity conservation. Some tools are: restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), microsatellite DNA, and also whole-genome SNP chips, DNA barcoding.

In this present study, we used the mitochondrial cytochrome c oxidase subunit I (COI) gene for the phylogenetic analysis in different camel breeds in India. There are two advantages to focus on COI gene (a) Universal primer; (b) COI shows a greater range of the phylogenetic signal. Compare to other genes which code protein, third position nucleotides of the COI gene show highly base substitutions, and therefore leading to the highest rate of molecular evolution, greater than 12S or 16S rDNA analysis [6]. The region of the COI gene gives low genetic variation within the species and significant

differences between the species. We identify negligible polymorphism between camels from South Africa and Sudan, which shows the origin of the Indian Camel. We are also reporting likelihood values, functional divergence in amino acid changes.

3. Materials and Methods

3.1 Animals resources

Four of the dromedary's indigenous camels were sampled for this experiment as shown in Table 1. They were Jaisalmeri (n= 3), Bikaneri (n=3), Kachchhi (n=3) and Mewari (n=3) from an in-house facility in National Research Centre on Camel (NRCC), Bikaner, Jorebeer, Rajasthan.

3.1.1 Animal sampling

Table 1: Sampling Summary for the Different Camel Population.

Population	Number of samples	Location	Type of analysis
Jaisalmeri	3	Jaisalmer, Barmer and Jodhpur region	Genetic diversity analysis
Bikaneri	3	Bikaner region	Genetic diversity analysis
Kachchhi	3	Gujrat region	Genetic diversity analysis
Mewari	3	Arawali mountain of the Rajasthan	Genetic diversity analysis

3.1.2 Collection of camel blood

Camel *(Camelus dromadaries)* blood samples were taken from jugular vein by 10 ml syringe. Blood samples were collected in EDTA coated test tubes at -20°C for long time storage.

3.1.3 DNA extraction and gel electrophoresis

Total genomic DNA was isolated from the collected blood of four different indigenous Camel breeds followed by the kit supplied by Invitrogen (cat no. K1820-01). Genomic DNA was measured by Nanodrop at 260 nm.

3.1.4 Polymerase chain reaction and gel electrophoresis

Universal primers of the mitochondrial cytochrome c oxidase subunit I (COI) were used to amplify the DNA targeted locus of the experimental samples [7,8]. PCR reaction was carried out in 50 µl reaction volume containing 10 X buffer without $MgCl_2$, 200 µM dNTPs, 0.5 µM of each primer (is presented in Table 2), 50 ng genomic DNA, 0.5 U of Taq polymerase and 2 mM $MgCl_2$. PCR machine was Applied Biosystem Thermo cycler and total 35 cycle was carried out. In each thermal cycle initial denaturation temperature was 95°C for 5 min followed by 95°C for 45 s, 62°C for 45 s, 72°C

for 45 s, and final extension was at 72°C for 10 min. Amplified samples were mixed with 6X DNA loading dye. 100 bp molecular markers were used as references to W. John Kress et al. 2012 and Mitchell et al. 2010. Run in 1% agarose gel for electrophoresis.

Table 2: Universal Primers of the Mitochondrial Cytochrome c Oxidase Subunit I (COI).

S. No.	Name of the Oligo	Sequence 5' to 3'	References
1	COI- F	TCTCAACCAACCACAAAGACATTGG	[7]
2	COI- R	TAGACTTCTGGGTGGCCRAARAAYCA	[8]

3.1.5 Gel elutes PCR products

Amplified PCR products was cut with the help of clean, sharp knife and was transferred to micro-centrifuge tube in eppendorf tube. Gel biding buffer was added according to instruction kit (MACHERY NAGEL; cat.no. 740609.50) and was kept in water bath. The temperature was maintained at 55˚C for 10 mints. The gel solution was mixed continuously, so that gel was completely melted. The whole solution was poured in a fresh column tube and centrifuged at 12, 000 rpm for 2 min at room temperature. Flow through was discarded and the column was washed with was buffer by centrifuge at 12,000 rpm for 2 min at room temperature. Flow through again was discarded and empty column was centrifuged at 12,000 rpm for 2 min at room temperature. Finally added around 30 µl hot elution buffer in to the centre of the column and centrifuged at 12, 000rpm for 2 min at room temperature. Elution buffer with binding DNA was collected in to new eppendorf tube and immediately stored at –20 ˚C.

3.1.6 Sequencing

For sequencing, PCR reaction mixture was contained RR big dye terminator mix (Big Dye terminator V 3.1; Thermofisher) 0.5 µl / sample, Sequencing buffer (50X) 1.5 µl / sample, Primer (Forward / Reverse – 20 µM), template was 50 ng, volume made up to 10 µl by adding MilliQ water followed by standardize cycle sequencing kit. PCR initial denature temperature was 95°C for 1 min followed by 95°C for 10 s, 55°C for 45 s, 60°C for 4 min, and final store was at 4°C for long time.

3.1.7 Purification

PCR amplified samples were purified by standard protocol. Samples were mixed with 2.5 µl of 125 mM EDTA, 100 µl of 100% molecular grade ethyl alcohol and was kept at -20°C for overnight. Next day, samples were centrifuged at 12,500 rpm for 20 min at 4°C. Supernatant was discarded and added 60 µl of 70% ethanol. Samples were centrifuged at 12,500

rpm for 20 min at 4°C. Supernatant was discarded and added 70% ethyl alcohol. Samples were again centrifuged at same speed and same temperature condition. After discarding supernatant, pellet was dried in air at room temperature. HiDi was added around 12 µl and samples were mixed thoroughly, incubated at room temperature for 10 min in dark place. Finally, samples were kept at 96°C for 5 min and immediately transferred in ice. Samples were preceded for sequencing (Applied Biosystem 3500×L-Genetic analyzer, 24 capillary).

4. Results

4.1 Genomic DNA solation of four different Camel breeds

Genomic DNA is isolated from four different Indian camels (*Camelus dromedarius*) at National Research Center on Camel, Jorbeer, Bikaneer, Rajasthan. DNA extraction is carried out using DNA extraction kit (Invitrogen). Concentration of the isolated DNA is measured by Nanodrops at 260/ 280 nm (Fig. 1).

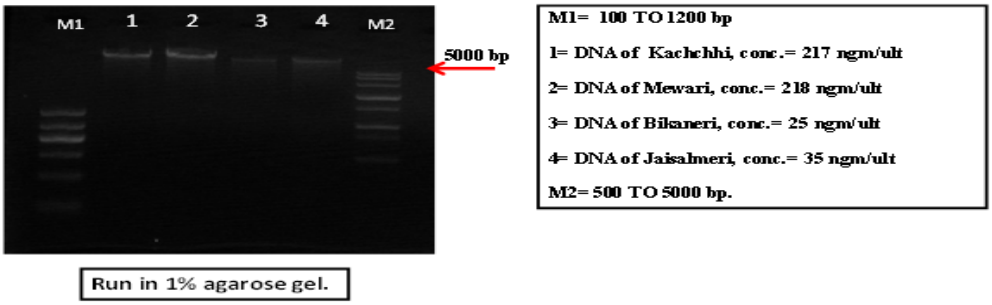


Fig. 1. Shows total genomic DNA isolation from camel blood samples.

Lane 1 shows DNA of Kachchchi camel, concentration is 217 ng/ µl. Lane 2 shows DNA of Mewari camel, concentration is 218 ng/ µl. Lane 3 shows DNA of Bikaneri, concentration is 25 ng/ µl. Lane 4 shows DNA of Jaisalmeri, concentration is 35 ng/ µl. M1 and M2 indicates molecular marker marker, 100 to 1200 bp and 500 to 5000 bp. DNA sample run in 1% agarose gel and analysis by using Gel Doc imager.

4.2 PCR amplification of mitochondrial cytochrome c oxidase subunit I (COI) gene

Compared to other genes which codes protein, third position nucleotides of the COI gene show highly base substitutions, and therefore leading to highest rate of molecular evolution. Size of the amplified COI gene is 710 bp (Figs. 2A and 2B).

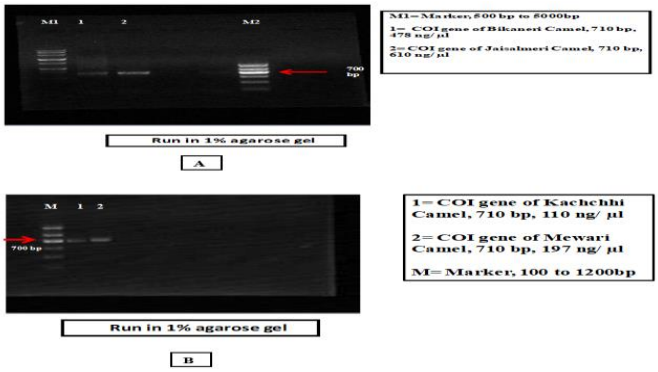


Fig. 2A and 2B. Shows amplified product of COI gene of four different camel blood.

Fig.2. A Lane 1 shows 710 bp amplified COI gene of Bikaneri Camel, concentration is 478 ng/µl and Lane 2 shows 710 bp amplified COI gene of Jaisalmeri Camel, concentration is 610 ng/µl. M1 and M2 indicates molecular weight marker, 500 bp to 5000 bp and 100 to 1200 bp. Fig.2B Lane 1 shows 710 bp amplified COI gene of Kachchhi camel, concentration is 110 ng/µl and Lane 2 shows 710 bp amplified COI gene of Mewari camel, concentration is 197 ng/µl. M indicates molecular weight marker, 100 to 1200 bp.

4.3 Sequence analysis

We have done Multiple Sequence Alignment (MSA) to find homology and the evolutionary relationships between four types of camel sequences. We have used Clustal Omega because it is the most used of all the Clustal tools. We have copied all the four types of camel DNA sequences in the Clustal Omega webserver. After a few seconds, we got the results from the webserver [1].

MSA results showed that the DNA sequences of Kachchhi, Mewari and Jaisalmeri are very similar but the DNA sequences of Bikaneri camels have some differences with the other three types of camels.

Percentage identity matrix results show that Mewari type has 96.61 % and 95.87% similarity with Kachchhi and Jaisalmeri, respectively. Bikaneri type has low similarity with Mewari, Kachchhi and Jaisalmeri with 46.92%, 47.17 % and 48.17 %, respectively (Table 3).

The result of the phylogenetic tree has shown the evolutionary relationships among four types of camels. The length of the branches of the phylogenetic tree has shown the genetic distance. Length of the branches has are similar with Kachchhi, Mewari and Jaisalmeriare the same and for Bikaneri, the length and brach are different (Fig. 3).

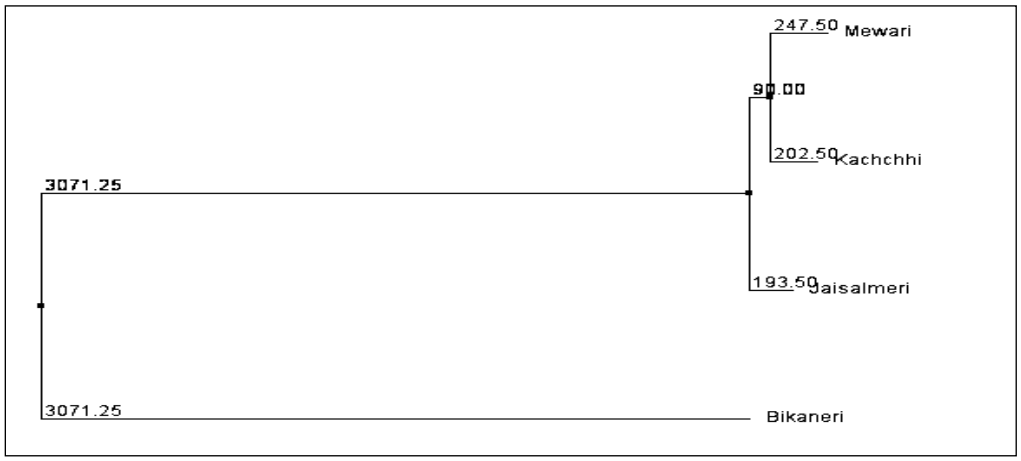


Fig. 3. Phylogenetic tree constructed based on COI genes of four different types of Indian camel breeds. All COI gene sequences were aligned and tree was constructed by neighbor joining methods. Mitochondrial COI genetic distance reveals close relationship between Mewari and Kachchhi. Phylogram also shows distance between Bikaneri and others are large.

The sequence alignment result has shown that Mewari, Kachchhi and Jaisalmeri have similar ATGC structures, but Bikaneri has some differences. Inserting gaps in the sequences has allowed MSA algorithm to match more with the other three types of camel DNA sequences. Most of the similarity observed in Thymine (T) region (Fig 4).

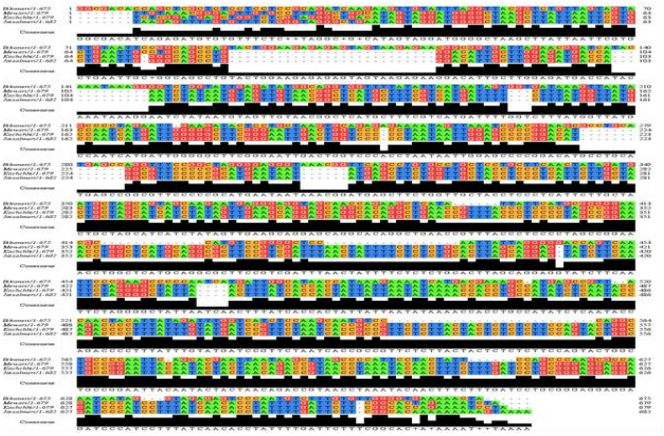


Fig. 4. Sequence alignment of four different breeds of Indian Camel (*Camelus dromedarius*).

Sequence analysis shows percentage of identity between Mewari and Bikaneri is 37%, whereas Kachchhi and Bikaneri is also 37%, Jaisalmeri and Bikaneri is 38 %. Green blocks indicate differences in nucleotide sequences and yellow bar summerizes similarity among the sequences in four breeds. Blue bar indicates weakly conserved residues.

The prediction of the nucleotides, when translated as cDNA has shown probable amino sequences of the four types of camels (Fig. 5).

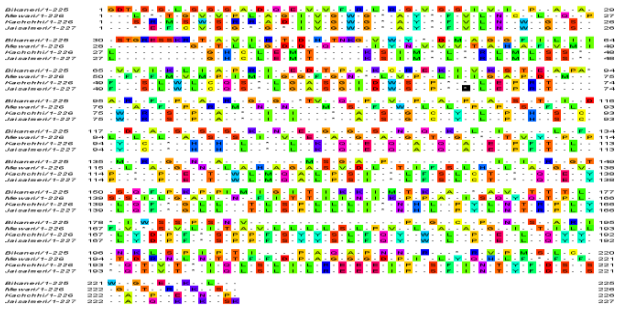
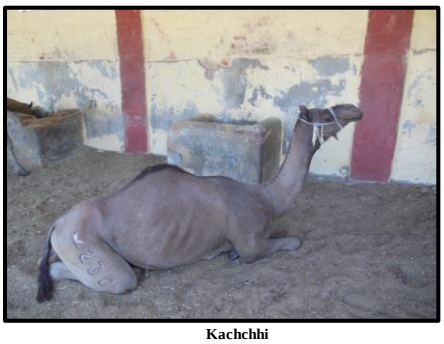


Fig. 5. Multiple sequence alignment of COI gene of four breeds of Indian Camel (*Camelus dromedarius*). Multiple sequence alignment of cDNA when translated from DNA.



Kachchhi

Fig. 6. Adult male camel of Kachchhi breeds.

Kachchhi:

TCTCGGATGAGCTGGTCTCGTAGGGCTGACATAGTAGGATGGGGCTAAGCTTATTAA
TTCGTGCTGAATTGGGGCAGCCTGGGACATTGCTTGGAGATGACCAAATCTATAATG
TAGTTGTAACGGCTCATGCTTTCGTCATGATTTTCTTTATGGTTATGCCAATCATGAT
TGGGGGCTTCGGGAATTGACTGGTCCCCCTAATAATTGGAGCCCCGGACATGGCGTT
CCCCCGCATGAATAATATGAGCTTCTGGTTGCTACCTCCCTCATTCTTGCTACTGCTA
GCATCATCTATAGTTGAAGCAGGAGCAGGCACAGGCTGAACCGTTTACCCTCCCCTA
GCCGGAAACCTGGCTCATGCAGGCGCTTCCGTCGATTTAACTATTTTCTCTCTGCACT
TAGCAGGAGTATCTTCAATTCTAGGGGCTATTAACTTTATCACCACTATTATTAATAT
AAAACCACCTGCCATATCTCAATACCAGACCCCTTTATTTGTATGATCCGTTCTAATC
ACCGCCGTTCTCTTACTACTCTCTCTTCCAGTACTGGCTGCCGGAATTACAATACTAC
TAACAGACCGTAACCTAAATACAACTTTCTTTGATCCTGCGGGAGGAGGAGATCCCA
TCCTTTATCAACACCTATTTTGATTCTTCGGCACCAGAAAATCCTA

Fig. 7. Cytochrome C Oxidase subunit I nucleotide (COI) sequence of Kachchhi breeds.

Mewari

Fig. 8. Adult male camel of Mewari breeds.

Mewari:

TTGTAAACGGGAGTTGTGCCTTTGGCTGGCATAGTAGGATGGGGCTAAGCTTATTAA
TTCGTGCTGAATTGCCTGCAGCCTGGGACATTGCTTGGAGATGACCAAATCTATAAT
GTAGTTGTAACGGCTCATGCTTTCGTCATGATTTTCTTTATGGTTATGCCAATCATGA
TTGGGGGCTTCGGGAATTGACTGGTCCCCCTAATAATTGGAGCCCCGGACATGGCGT
TCCCCCGCATGAATAATATGAGCTTCTGGTTGCTACCTCCCTCATTCTTGCTACTGCT
AGCATCATCTATAGTTGAAGCAGGAGCAGGCACAGGCTGAACCGTTTACCCTCCCCT
AGCCGGAAACCTGGCTCATGCAGGCGCTTCCGTCGATTTAACTATTTTCTCTCTGCAC
TTAGCAGGAGTATCTTCAATTCTAGGGGCTATTAACTTTATCACCACTATTATTAATA
TAAAACCACCTGCCATATCTCAATACCAGACCCCTTTATTTGTATGATCCGTTCTAAT
CACCGCCGTTCTCTTACTACTCTCTCTTCCAGTACTGGCTGCCGGAATTACAATACTA
CTAACAGACCGTAACCTAAATACAACTTTCTTTGATCCTGCGGGAGGAGGAGATCCC
ATCCTTTATCAACACCTATTTTGATTCTTCGGCACTAGAAAATCTA

Fig. 9. Cytochrome C Oxidase subunit I nucleotide (COI) sequence of Mewari breeds.

Bikaneri

Fig. 10. Adult male camel of Bikaneri breeds.

Bikaneri:

GGCGACACCAGCTCGCTCTCCTCCTCCGCAGATCAAGAAGTTGTATTTAGGTTACGG
TCTGTTAGTAGTATTGTAATTCCGGCAGCCAGTACTGGAAGAGAGAGTAGTAAGAG
AACGGCGGTGATTAGAACGGATCATACAAATAAAGGGGTCTGGTATTGAGATATGG
CAGGTGGTTTTATATTAATAATAGTGGTGATAAAGTTAATAGCCCCTAGAATTGAAG
ATACTCCTGCTAAGTGCAGAGAGAAAATAGTTAAATCGACGGAAGCGCCTGCATGA
GCCAGGTTTCCGGCTAGGGGAGGGTAAACGGTTCAGCCTGTGCCTGCTCCTGCTTCA
ACTATAGATGATGCTAGCAGTAGCAAGAATGAGGGAGGTAGCAACCAGAAGCTCAT
ATTATTCATGCGGGGGAACGCCATGTCCGGGGCTCCAATTATTAGGGGGACCAGTCA
ATTCCCGAAGCCCCCAATCATGATTGGCATAACCATAAAGAAAATCATGACGAAAG
CATGAGCCGTTACAACTACATTATAGATTTGGTCATCTCCAAGCAATGTCCCAGGCT
GCCCCAATTCAGCACGAATTAATAAGCTTAGCCCCATTCCTACTATTCCAGCCCAAG
CGCCAAATAATAGGTAGAGAGTCCCAATGTCTTTGTGTTGGGGTGAAAAACTA

Fig. 11. Cytochrome C Oxidase subunit I nucleotide (COI) sequence of Bikaneri breeds.

Jaisalmeri

Fig. 12. Adult male camel of Jaisalmeri breeds.

Jaisalmeri:

TAAGAGTTTTGTGTTTCTGGGTAGGGCTGAATAGTAGGATGGGGCTAAGCTTATTAA
TTCGTGCTGAATTGGGGCAGCCTGGGACATTGCTTGGAGATGACCAAATCTATAATG
TAGTTGTAACGGCTCATGCTTTCGTCATGATTTTCTTTATGGTTATGCCAATCATGAT
TGGGGGCTTCGGGAATTGACTGGTCCCCCTAATAATTGGAGCCCCGGACATGGCGTT
CCCCCGCATGAATAATATGAGCTTCTGGTTGCTACCTCCCTCATTCTTGCTACTGCTA
GCATCATCTATAGTTGAAGCAGGAGCAGGCACAGGCTGAACCGTTTACCCTCCCCTA
GCCGGAAACCTGGCTCATGCAGGCGCTTCCGTCGATTTAACTATTTTCTCTCTGCACT
TAGCAGGAGTATCTTCAATTCTAGGGGCTATTAACTTTATCACCACTATTATTAATAT
AAAACCACCTGCCATATCTCAATACCAGACCCCTTTATTTGTATGATCCGTTCTACCG
CCGTTCTCTTACTACTCTCTCTTCCAGTACTGGCTGCCGGAATTACAATACTACTAAC
AGACCGTAACCTAAATACAACTTTCTTTGATCCTGCGGGAGGAGGAGATCCCATCCT
TTATCAACACCTATTTTGATTCTTCGGCACAAAAAAAAAGTAAAA

Fig. 13. Cytochrome C Oxidase subunit I nucleotide (COI) sequence of Jaisalmeri breeds.

4.4 Percent Identity Matrix - created by Clustal 2.1

The percentage identity matrix of COI gene of four different breeds [Figs. 7, 9, 11, and 13] in Indian Camel (Camelus dromedarius) was constructed by Clustal 2.1 version as indicated (Table 3).

Table 3: Signifies the Percentage identity Matrix of Four Different Breeds of Indian Camel *(Camelus dromedarius).*

Bikaneri	**100.00**	**46.92**	**47.17**	**48.17**
Mewari	46.92	100.00	96.61	95.87
Kachchhi	47.17	96.61	100.00	96.32
Jaisalmeri	48.17	95.87	96.32	100.00

Table 3 shows Bikaneri and Mewari breeds are 46.92% similar, whereas Kuchchhi and Mewari are around 96.61% similar. Identity matrix also shows Kuchchhi and Bikaneri are 47.17% similar. Result shows Jaisalmeri and Kachchhi are 96.32% similar, Jaisalmeri and Mewari are 85.87% similar whereas Jaisalmeri and Bikaneri are 48.17% similar.

4.5 Phylogenetic Tree

Phylogenetic tree was constructed based on the variation of the COI genes in four different breeds in Indian Camel (Fig. 3).

5. Discussion

In this present study, mitochondrial COI gene was used to determine the genetic variation of four breeds of Indian Camel – Bikaneri, Jaisalmeri, Mewari, and Kuchchhi. We have successfully isolated genomic DNA from four different Indian camel breeds (*Camelus dromedarius*) Approximately 709 bp length of mitochondrial cytochrome oxidase gene was amplified using PCR. The specificity of amplified PCR products was verified by sequencing of DNA amplicon. It was reported early, mitochondrial DNA genome has higher incident of substitution than nuclear DNA to identify similarity related individuals [9]

Each DNA barcoding marker of COI genes were well separated by presenting distinct distant clades in between four breeds. Mewari, Kachchhi and Jaisalmeri have shown highest close distance (Fig. 3). Phylogenetic tree also reveals long distance between Bikaneri and others.

Phylogenetic data are displayed with interspecific sequence variation of four breeds in Indian Camel, which is well supported by sequence alignment using Clustal 12.1. Phylogenetic tree was drawn with appropriate scale which infers the evolutionary distances in same species.

In order to correlation among four breeds [Figs. 6, 8, 10, and 12] in Indian Camel (Camelus dromedarius), Fig. 4 demonstrates that Bikaneri breeds are around 37 % identical with Mewari and Kachchhi. Sequence alignment data also shows Jaisalmeri camel breed are 38 % similar with Bikaneri camel breed. All sampling was done in (NRCC) National Research Centre On Camel, Jorebeer, Bikaneer, Rajasthan, India.

We noticed that variation of COI sequences in four breeds in Indian Camel using Clustal O (1.2.4) multiple sequence analysis based on insertion, deletion, substitution (Fig. 5). We have found variation of nucleotide distribution within the codons across the four breeds of Indian Camel [10].

Based on the above data analyze, it is believed that incredible mutation rate in the individual breed in India and divergent from the main species. It is also believed that environmental and geographical parameter including biotic potential is the main reason to make rapid polymorphic changes in mitochondrial DNA genome. There are two acceptable reports that are mostly proved in our present study. Circulation of the camel breeds across the geographic area and another is the evolutionary divergence advices us all breed are related to same progenitor during the developmental history.

5. Acknowledgment

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6. Conflicts of Interest

The authors declare there is no conflicts of interest.

7. Authors' Contribution

PP^{1a} performed molecular biology experiment, assays, analyzed data and drafted manuscript. PP^{1b} performed some of the molecular biology assays. ERB initiated the project, designed the whole experiments, analyzed the data and wrote the manuscript.

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