Genetic diversity analysis among Indian goat breeds based on mitochondrial DNA

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Received: 24 July 2019; Accepted: 24 October 2019

ABSTRACT

India ranks second in goat population with 34 genetically recognized and registered breeds. Information on their diversity and origin and ancestry is little known. Hence, the mtDNA based genetic diversity analysis of Indian goats; targeting mitochondrial HVR1 region from 21 Indian breeds belonging to different geographical regions was undertaken. A total of 124 haplotypes were identified and haplotype diversity estimate ranged from 0.67 to 1.0 with an average value of 0.99. The average nucleotide diversity was minimum (0.02) in Kanniadu and maximum in Surti breeds. Analysis of molecular variance revealed 5.16% variation among the breeds and 94.84% within breeds indicating weak phylogeographic structure. Neighbor-joining tree analysis revealed that the maximum number of individuals of Indian goats fall under A and few in B and C lineages. Principal component analyses of the Indian goat breeds revealed that Kanniadu goats clustered distantly from rest of the breeds of the country. Mantel test revealed a significant correlation between FST and geographical distance (r=0.29) among the goat breeds. The mismatch distribution analysis of the Indian goat breeds revealed bimodal distribution patterns. The analysis revealed that Kanniadu is highly distinct from the rest of the breeds.

Keywords: Diversity, Genetic, Goat, Haplotype, mtDNA

Goat commonly known as ‘poor man’s cow’ (MacHugh and Bradley 2001) has a total population of 1,034 million in the world and out of which 133 million are in India (FAOSTAT 2017). The origin of the domestic goat is uncertain and controversial being the first domesticated animal during Neolithic era in the Fertile Crescent region (Porter 1996, Pringle 1998) is commonly reared for meat, milk, wool, and skin. Earlier studies had reported a second domestication event of Kashmiri breeds in Pakistan (Porter 1996, Meadow 1996). Along with the domestication events, at least two wild species of Capra (Shackleton 1997) have been suggested contributing to the gene pool of domestic goats (Clutton-Brock 1981). Recent advances in the field of molecular genetics enable us to gain more knowledge about the origin and diversity of the goat breeds. Mitochondrial DNA (mtDNA) inherited from the maternal side does not involve in recombination process but undergoes high mutation rate than nuclear DNA. Hyper Variable Region 1 (HVR1) in the mitochondrial sequence is highly polymorphic than the other regions in mitochondrial DNA. Earlier studies reported seven maternal lineages (A, B, C, D, E, F and G) (Luikart et al. 2001, Sultana et al. 2003, Joshi et al. 2004, Sardina et al. 2006, Naderi et al. 2007) of domestic goats and the later studies revealed lineage E of Indian goats to be subtype of lineage A.

At present six different maternal lineages are present in the world’s goat population. The mtDNA diversity and phylogenetic relationship of North and South Indian goat breeds were studied by Joshi et al. (2004) and Kamalakkanan et al. (2018). No exhaustive study has been conducted covering most of the Indian goat breeds. Hence, the present study aimed at analyzing 21 well-defined goat populations belonging to different agro-climatic zones of India to provide a comprehensive view of the genetic diversity of Indian goat breeds and their phylogenetic relationship with different goat lineages of the world.

MATERIALS AND METHODS

Sample collection: Blood samples (N=192) were collected randomly from 21 Indian goat breed population (Zalawadi, Surti, Sirohi, Sangamneri, Osmanabadi, Mehsana, Marwari, Malabari, Kutchi, Kanniadu, Jharkhand Black, Jamunapari, Jakhana, Gohilwadi, Ganjam, Gadi, Chegu, Changthangi, Black Bengal, Beetal and Barbari) from the different geographical regions of India. It was ensured that sampled animals were not related to each other and while collecting proper care was taken to distinguish the pure breed from crossbred animals.

DNA isolation and PCR amplification: The genomic DNA was isolated using a standard procedure. Primer reported by Naderi et al. (2008) [CAP-F (5’-CGTGTATGCAAGTACATTAC-3’) – CAP-R (5’-CTGATTAGTCATTAGTCATTACCATC-3’)] was used for amplifying the HVR1 region in all the Indian goat breeds. The PCR amplification
was carried out in a volume of 25 µl, containing 100 ng genomic DNA, 0.5 µl of each primer, 0.3 µl of Taq DNA polymerase (Fermentas), 0.5 µl MgCl₂ and 0.5 µl of dNTPs. The PCR protocol was standardized with following steps; initial denaturation at 95°C for 5 min, followed by 30 cycles 95°C for 30 sec, annealing temperature at 60°C for 30 sec extension at 72°C for 30 sec, final extension at 72°C for 10 min and remaining at 4°C.

Mitochondrial DNA sequence analyses: The amplified product (598 bp) was visualized by Agarose gel electrophoresis and custom DNA sequencing was carried out. The nucleotide data obtained after editing (464 bp) was submitted to the Genbank (JX271778 – JX271587) and was used for the present analysis. Arlequin software was used for calculation of the number of haplotypes, haplotype diversity, nucleotide diversity, mean number of pairwise differences, number of polymorphic sites and Fₛᵀ values, etc were calculated as described by Excoffier and Lischer (2010). Mismatch distribution analysis was carried out using DnaSP (Rozas et al. 2017). Principal Component Analysis (PCA) by using GenAIEx (Peakall and Smouse 2012). Mantel test was carried out using GenAIEx. For neighbor-joining tree and Median Joining tree analyses, published wild goat sequences (EF989163, EF989164, EF989165, and EF989166) and sequences of published maternal lineages, A, B, C, D, F and G (KR059184, KR059219, GU229280, KR059210, KR059226 and KR059213) were included. NJ tree was constructed with MEGA X (Kumar et al. 2018) using the Kimura-2 parameter model with a bootstrap value of 2000. The median-joining tree was constructed using NETWORK 5 software (Bandelt et al. 1999).

RESULTS AND DISCUSSION

The edited 464 bp mtDNA HVR1 sequences of 192 Indian goats were analyzed and the number of haplotypes, haplotype diversity, nucleotide diversity, mean number of pairwise differences, number of polymorphic sites was calculated (Table 1).

Nucleotide and Haplotype diversity: Nucleotide and haplotype diversity are two important parameters for assessing genetic difference and polymorphism at the
population level. A total of 124 haplotypes ranging from 2 (Chegu) to 12 (Gohilwadi) in different goat breeds were identified. A total of 38 shared haplotypes was found out of which 14 were shared between different breeds. Haplotype diversity was minimum in Kamniadu and Chegu (0.6667±0.1409; 0.6667±0.3143) and maximum in Zalawadi, Sangamneri, Mehsana, Kutchi, Jharkhand Black, Jakhrana, Ganjam, Gaddi (1.0000±0.5000). Nucleotide diversity was minimum in Kanniadu (0.004702±0.003148) and maximum in Surti (0.033340±0.018051). Though the sample size varied in the present study and in work done by Joshi et al. (2004), the average haplotype diversity remained the same, i.e. 0.993 vs 0.938, indicating higher diversity. The number of haplotypes identified in the present investigation was 124 from 192 animals, which is comparatively higher than 200 from 363 animals (Joshi et al. 2004) and 78 from 104 animals (Kamalakannan et al. 2018).

The haplotype diversity of the breeds ranged from 0.9524±0.0403 (Malabari) to 0.9921±0.0154 (Kamniadu), and it also varied in the other Indian breeds (Kamalakannan et al. 2018). However, the haplotype diversity of the breeds remained the same in the Indian breeds studied by Joshi et al. (2004). In this study, we found haplotype diversity of goat breeds varying from 0.8889±0.0596 in Malabari to 0.6667±0.1409 in Kamniadu. The above comparison may be an indication of increased haplotype diversity in South Indian goat breeds. The average haplotype diversity in Indian goat breeds has been found to be 0.9927 whereas it was 0.9829 and 0.9333 in Chinese goat breeds reported by Zhao et al. (2011) and Liu et al. (2006). The haplotype diversity between the Indian and Chinese goat breeds was found to be comparable and is at par with each other irrespective of their geographical isolation. This could be due to the fact that both the Indian and Chinese goat breeds are from the same lineage A (Joshi et al. 2004, Liu et al. 2006, Kamalakannan et al. 2018). Study done in European goat breeds by Hoda et al. (2014) indicated that in 6 Albanian goats, the average nucleotide diversity was 0.036 and average haplotype diversity was 0.996. Similar findings were also reported by Sardina et al. (2006) in three Sicilian goat breeds with nucleotide diversity of 0.024 and haplotype diversity of 0.969. The high haplotype diversity and low nucleotide diversity of the population probably may be due to the expansion after the population has undergone bottleneck (Su et al. 2014). A population with a high haplotype diversity value (Hd=0.5) and low nucleotide diversity (Pi < 0.005 or 0.5%) indicated that it might have experienced a bottleneck effect (Grant and Bowen 1998).

Moreover, high haplotype diversity and lower nucleotide diversity suggested that the time after population expansion was long enough to examine the change in haplotypes that resulted from mutation, but it was not long enough to accumulate big differences among sequences (Avise 2000).

**Transition vs Transversion:** In the 464 nucleotide sequence obtained from 192 individual animals, there were 606 polymorphic sites which were mostly single-nucleotide substitutions or deletions. The minimum number of polymorphic sites was in Jakhrana and Chegu (9) and maximum recorded in Barbari (52). The mean number of substitutions in the studied Indian goat breeds was 29 and that of the transitions and transversion was 28.381 and 0.619 respectively. The overall ratio of transition vs transversion was 45.84 which revealed the transition bias in Indian goats.

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**Table 1. mtDNA diversity parameters among Indian goats**

| Breed             | No. of samples | No. of haplotypes | Haplotype diversity | Nucleotide diversity | Mean number of pairwise difference | No. of polymorphic sites |
|-------------------|----------------|-------------------|---------------------|----------------------|-----------------------------------|--------------------------|
| Zalawadi          | 4              | 4                 | 1.0000±0.1768       | 0.018319±0.012840    | 8.500000±4.989274                | 16                       |
| Surti             | 12             | 8                 | 0.9242±0.0575       | 0.033340±0.018051    | 15.469697±7.439034               | 39                       |
| Sirohi            | 13             | 7                 | 0.7949±0.1091       | 0.018678±0.010373    | 8.6666±4.281730                  | 31                       |
| Sangamneri        | 11             | 11                | 1.0000±0.0388       | 0.024925±0.013488    | 11.272727±5.547349               | 42                       |
| Osmanabadi        | 10             | 7                 | 0.8667±0.1072       | 0.014751±0.008583    | 6.8444±4.521303                  | 21                       |
| Mehsana           | 2              | 2                 | 1.0000±0.5000       | 0.021552±0.022604    | 10.00000±7.416198                 | 10                       |
| Marwari           | 10             | 6                 | 0.8889±0.0754       | 0.017672±0.010132    | 8.200000±4.156941                | 21                       |
| Malabari          | 10             | 5                 | 0.8889±0.0596       | 0.030651±0.017001    | 14.222222±6.975323               | 38                       |
| Kutchi            | 10             | 10                | 1.0000±0.0447       | 0.023563±0.013252    | 10.93333±5.436856                | 40                       |
| Kamniadu          | 12             | 5                 | 0.6667±0.1409       | 0.004702±0.003148    | 2.181818±1.297376                 | 10                       |
| Jharkand Black    | 6              | 6                 | 1.0000±0.0962       | 0.016379±0.012921    | 7.600000±4.135215                 | 17                       |
| Jamunapari        | 13             | 11                | 0.9744±0.0389       | 0.022104±0.012136    | 10.256410±5.009673                | 42                       |
| Jakhrana          | 2              | 2                 | 1.0000±0.5000       | 0.019397±0.020446    | 9.000000±6.708204                 | 9                        |
| Gohilwadi         | 13             | 12                | 0.9744±0.0389       | 0.015805±0.008892    | 7.3333±4.670610                   | 22                       |
| Ganjam            | 8              | 8                 | 1.0000±0.0625       | 0.030172±0.017286    | 14.000000±7.041163                | 44                       |
| Gaddi             | 3              | 3                 | 1.0000±0.2722       | 0.015805±0.012707    | 7.3333±4.727495                   | 11                       |
| Chegu             | 3              | 2                 | 0.6667±0.3143       | 0.012931±0.010558    | 6.000000±3.927922                 | 9                        |
| Changthangi       | 11             | 9                 | 0.9455±0.0659       | 0.026920±0.014861    | 12.490909±11.62215                | 47                       |
| Black Bengal      | 14             | 9                 | 0.9341±0.0448       | 0.032280±0.017256    | 14.978022±7.132654                | 45                       |
| Beetal            | 11             | 9                 | 0.9455±0.0659       | 0.022100±0.012339    | 10.25454±5.070595                 | 40                       |
| Barbari           | 14             | 11                | 0.9670±0.0366       | 0.026620±0.014365    | 12.351648±5.937884                | 52                       |
| Total             | 192            | 124               | 0.9927              | 0.02351              | 10.90789                          | 606                      |
between FST and geographical distance \( r=0.29 \) \( P<0.031 \). The test revealed a significant correlation relationship between geographic distance and genetic divergence. The test substantiated the overall structuring of the goat population and was in agreement with the findings of Joshi et al. (2004) and Luikart et al. (2001).

**Analysis of molecular variance:** AMOVA analysis revealed variation among breeds (5.16%) as well as within breed (94.84%) (Table 2). Higher variation within the Indian goat breeds may partly be due to lack of selection pressure applied and large effective population size as observed by Joshi et al. (2004) and Araimi et al. (2017).

**Mantel test:** Mantel test was performed to evaluate the relationship between geographic distance and genetic divergence. The test revealed a significant correlation between \( F_{ST} \) and geographical distance \( r=0.29 \) \( P<0.031 \) among the Indian goat breeds. Kanniadu breed (South India) clustered separately from rest of the Indian goat breeds.

**Population genetic structure:** Neighbor-joining tree revealed 3 different clusters wherein the maximum number of goats fallen in Lineage A along with the wild goat EF 989165, that can be assumed to be the ancestor of Lineage A. Few of the goats also fallen under lineages B and C. None of the Indian goat fallen under the lineages D and G. Only the wild goat (AJ 317866) got clustered in Lineage F (Fig. 1). To further know more about the phylogenetic relationship among Indian goat breeds, we performed the MJ network analysis and the results showed two clusters with the maximum in haplogroup A and few in haplogroup B (Fig. 2).

The present study indicated that the maximum number of Indian goat breeds fallen in lineage A, and it is in agreement with the findings of Kamalakannan et al. (2018). Naderi et al. (2007) also reported that a maximum number of individuals in the goat population of the world lied in Lineage A while studying 2,430 individuals from all over the world. Moreover, the lineages identified in the present study had also been reported by Naderi et al. (2007). Joshi et al. (2004) also reported that Lineage A was present in the goat breeds of all the continents. The presence of lineage D had been reported by Sultana et al. (2003) in the Pakistani and Chen et al. (2005) in the Chinese goat breeds.

**Genetic differentiation:** The genetic differentiation \( F_{ST} \) values in Indian goats ranged between 0.000 in many of the breeds whereas it is maximum between Kanniadu and rest of the breeds (0.56792). Deviation of \( F_{ST} \) from zero suggested weak population subdivision in Indian goat breeds (Zalawadi, Surti, Sirohi, Sangamneri, Osmanabadi, Mehsana, Marwari, Kutchi, Malabari, Kanniadu, Jharkhand Black, Jamunapari, Jakhani, Gohilwadi, Ganjam, Gaddi, Chegu, Changthangi, Black Bengal, Beetal, Barbari) which is due to constant gene flow among the breeds. PCA showed a maximum variance of 80.07 by first 3 coordinates. PCA of the Indian goat breeds revealed that Kanniadu goats of Southern-India clustered distantly from the other Indian goats as it has the minimum nucleotide and haplotype diversity.

**Mismatch distribution:** The mismatch distribution chart for the Indian goat breeds revealed bimodal mismatch distribution patterns containing two major peaks (with maximum values) at 9 and 29, pairwise differences with smooth curve suggesting the expansion of goat population (Fig. 3). Moreover, the mismatch distribution pattern contains one peak at 9 pairwise differences in the Lineage A of Indian goats. The similar type of results of bimodal distribution had also been reported in Omani goats. These peaks were similar to the peaks identified previously for domestic goats throughout the Old World (Naderi et al. 2007, Zhao et al. 2014, Kibegwa et al. 2016).

The present comprehensive study revealed substantial mtDNA diversity among the Indian goat breeds. But there is weak population subdivision in Indian goat breeds. Most of the Indian goats were clustered in lineage A with only few clustering in lineage B and C. Among the Indian goat breeds, Kanniadu breed (South India) clustered distantly.
from rest of the breeds.

ACKNOWLEDGEMENTS

The authors duly acknowledge the farmers for their kind consent to take blood samples of their animals. The author also expresses their thanks to Director, ICAR-NBAGR, for providing facilities to carry out this work. The facilities and support provided by the ICAR-NDRI are greatly acknowledged.

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