ABSTRACT: The mitochondrial hypervariable (HVI) region sequence of 93 goats belonging to Khari, Chyangra, Terai and Sinhal breeds from different part of the country were partially sequenced to examine the phylogenetic relationship of Nepalese goat breeds. High mtDNA diversity among Nepalese goat breeds was observed and all goat haplotypes could be classified into four haplogroups (A-D). It appeared that mitochondrial lineage/ haplogroup A was observed in most of the Nepalese goats whereas only one breed (Chyangra) contains all four haplogroups. These sequences were compared with published sequences of domestic goats of neighboring countries (Bhutan, India, Pakistan and China) goats to determine the relationship of Nepalese goats among goat resources of the region. The study also revealed that the gene flow among the neighboring countries.

Keywords: Goat; Hypervariable (HVI) region; Mitochondrial DNA; Nepalese indigenous breeds

Introduction

Nepal has a sizeable indigenous goat population with four identified breeds and many non-descript goats. Identified goat breeds have been characterized in phenotype (Neopane et al. (2005); Khanal et al. (2005)) and karyotype (Rasali et al. (1998)). Terai goat is well-adapted to the topography and climate of Terai region (<610m asl). Khari goat, a prolific meat breed, is well-adapted to mid hill region (610-4877m asl) throughout the country from east to west. Chyangra and Sinhal are adapted to the high hills (>4877m asl). There are several hypotheses about the origin of Nepalese goats. According to hair quality, the Chyangra goat probably came from Cashmere goat of Pakistan (Sultana et al. (2003)) and from other Himalaya regions of the continent. The goat breeds in high hills (Himalayan region) of Nepal such as Chyangra goat and Sinhal goats might also have genetic introgression from Tibet, China as Nepalese goat and sheep flock of these areas share common pasture with Tibetan flocks as they follow migratory system to escape the extreme cold during winter (Gorkhali et al. 2001)). However, the goat breeds in mid hills (Khari goat) and lowland (Terai goat) probably influenced by Indian goat breeds as countries share free border and also due to the geographical structure in these areas have easier accessibility in comparison to the high altitude areas. Apart from biochemical study on Khari goat (Kunwar et al. (2000)) which reveals three distinct strains within the population, a little attention has been paid to the genetic diversity studies of these indigenous goat resources. There is, therefore, a need for an extensive study of Nepalese goat breeds to understand their phylogenetic structure. The main objective of this study was to examine genetic diversity and phyleography of six different Nepalese goat populations based on the analysis of mtDNA D-loop hypervariable (HVI) region. Ninety three individuals from all six populations of Nepalese indigenous goat populations from different geographic regions were sequenced at mitochondrial HVI region. A comprehensive analysis of collected molecular and geographical data was conducted to address the issues regarding genetic diversity, and distribution of mitochondrial lineages (haplogroups) among Nepalese goats. Further comparisons with other goat populations in neighboring countries were also carried out.

Materials and Methods

Population sampling. Ninety three blood samples were collected from six populations of indigenous goats which were true to type with the phenotypic characteristics and unrelated with each other.

DNA amplification and sequencing. Six hundred twenty five bp long Mitochondrial DNA HVI region of goats was amplified using the primers: forward 5’ CATTACACCGTCGCTAC 3’ and reverse 5’ GGGCTGTAGTGCGAT 3’ (Wu, Y.P. et al., 2009). PCR amplification was carried out in 50 µl reaction mixtures including each primer (1µl of a 10 umol/L solution), dNTPs (4 µl of a 2.5 mmol/L solution), 5 µl of 10X buffer and 1.5 µl of 5U/µl Taq DNA polymerase (Tiangen Biotech, Beijing, China). The PCR conditions were an initial denaturing step at 95°C for 5 minutes followed by 35 amplification cycles (94°C for 30s, 56.2°C for 30s and 72°C for 30s) and a final extension at 72°C for 10 min in a Programmable Thermal Controller.

Analysis of sequence data. The sequences were edited using Chromas version 2.23. Included in the analysis were published mtDNA HVI region genebank of domestic goats from India, Pakistan, China and Bhutan. Sequences were aligned by the Cluster W method included in the program MEGA 4.0 (Tomura et al. (2007)). Sequences variation was exported by using MEGA 4.0. Twenty two goat mtDNA control region reference sequences belonging to the six known haplogroups/ lineages that were recommended by Naderi et al. (2007) were also included in our analysis, to facilitate the recognition of haplogroup status of each individual. Neighbor-joining (NJ) tree was constructed for these mtDNA HVI region
sequences using the program Mega 4.0. The robustness of internal branches was estimated based on 1000 bootstrap replications. Median Joining (MJ) network (Bandelt et al. (1999)) was constructed by using Network 4.2 (http://www.fluxus-engineering.com/sharenet.htm), in which transitions, transversions, and insertions/deletions were equally weighted. Hapлотype diversity (h) and nucleotide diversity (π) (Kamura et al. (2004)) for each goat breed/population were also estimated by using DNASP 4.10 (Rozas et al. (2003)).

Results and Discussion

Sequence variation. The mtDNA control region HVI sequences from all 93 goat samples were highly polymorphic with 102 variable sites over the 625 bp of the alignment. Out of total variable sites, 100 variants were transitions and 2 variants were transversions, and there was no insertion/deletion.

Genetic diversity. The genetic diversity estimated based on the mtDNA HVI region sequences varied substantially among breeds (Table 1). The analysis of haplotype diversity and nucleotide diversity showed that Khari Ilam and Sinhal were the least variable group and chyangra was highly diverse group. When the goat samples were grouped according to the ecological distribution, the high hill area had the highest haplotype diversity (0.981±0.016) and nucleotide diversity (0.04±0.0051). There was not much difference among the populations of mid high and low land area.

### Table 1 Distribution of mtDNA haplogroups in Nepalese goat breeds/populations

<table>
<thead>
<tr>
<th>Breed/population (Breed code)</th>
<th>Ecological zone</th>
<th>No. of Individual</th>
<th>No. of haplotypes</th>
<th>Haplotype diversity (h±SD)</th>
<th>Nucleotide diversity (π±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High hill</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chyangra (NGCh)</td>
<td>28</td>
<td>23</td>
<td>0.981±0.016</td>
<td>0.040±0.005</td>
<td></td>
</tr>
<tr>
<td>Sinhal (NGS)</td>
<td>10</td>
<td>6</td>
<td>0.86±0.015</td>
<td>0.026±0.001</td>
<td></td>
</tr>
<tr>
<td>Khari (NGK)</td>
<td>45</td>
<td>29</td>
<td>0.973±0.011</td>
<td>0.027±0.001</td>
<td></td>
</tr>
<tr>
<td>Khari Bandipur (NGKB)</td>
<td>45</td>
<td>29</td>
<td>0.973±0.011</td>
<td>0.027±0.001</td>
<td></td>
</tr>
<tr>
<td>Khari Ilam (NGKI)</td>
<td>12</td>
<td>7</td>
<td>0.864±0.079</td>
<td>0.017±0.006</td>
<td></td>
</tr>
<tr>
<td>Khari Salyan (NGS)</td>
<td>19</td>
<td>13</td>
<td>0.942±0.037</td>
<td>0.032±0.006</td>
<td></td>
</tr>
<tr>
<td><strong>Low land</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Terai (NGT)</td>
<td>20</td>
<td>16</td>
<td>0.974±0.011</td>
<td>0.027±0.001</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>93</td>
<td>66</td>
<td>0.990±0.003</td>
<td>0.028±0.001</td>
<td></td>
</tr>
</tbody>
</table>

**Haplogroup classification and phylogenetic analysis.** Sixty six haplotypes were identified in 93 mtDNA HVI region sequences. Using the available goat mitochondrial (mtDNA) haplogroup classification system (Naderi et al. (2007)), four haplogroups (A, B, C and D) were found among indigenous Nepalese goats (Figure 1). Nepalese goat breeds was consistent with the world scenario described in previous studies (Liu et al. (2009); Sultana et al. (2003); Joshi et al. (2004); Chen et al. (2005); Wang et al. (2008)), in which haplogroups A and B are the main components of Nepalese goat. There was no specific haplogroup distribution pattern in breeds/populations or among the different ecological regions. One breed (Chyangra goat) contained all four haplogroups (A-D). Khari Bandipur goats contained haplogroups A, B and D. The other breeds/populations contained two haplogroups (A and B). When considered the ecological distribution, high hill regions contained all four haplogroups, mid hill regions contained three haplogroups (A, B and D) and low land regions contained only two haplogroups (A and B). The median joining network of 66 haplotypes of Nepalese goat (Fig. 2) also agrees the above statement as breeds from different geographic regions did not cluster together and separate from other regions. Some haplotypes were shared by individuals of different breeds from different geographical regions. In addition, most breeds from one geographical region distributed throughout the whole network.

**Fig. 1 Phylogenetic trees of 93 Nepalese Goat mtDNA HVI region sequences and 22 goat reference sequences.** The phylogenetic positions of the 22 reference sequences, which were defined by Naderi et al. (2007), were marked by black dots in the neighbor-joining tree. **Fig. 2 Network profiles of the four alogroups based on the mtDNA HVI region sequences.** Each circle represents a haplotype. The area is proportional to the sample size sharing that haplotype. The number of mutations differed between two haplotypes are shown between two circles.
Phylogeographic analysis. Considering the possibility of the intermixing of the indigenous goats with the goats of neighboring countries, Nepalese goat sequences were compared with goats from China, India, Pakistan and Bhutan. Khari Salyan and Terai goat sequences shared with Indian goats and Khari Salayan shared with Pakistani and Chinese goats indicating same origins or gene flow between goat populations (Table not shown). Chyangra demonstrate four haplogroups which is similar as the Tibetan goat (Lui et al. (2009)) as speculated. Haplotypes C are found in Chyangra goat (2 individuals) and D is found in Chyangra goat (2 individuals) and Khari Bandipur (1 individual). These haplogroups are found in Western China: Tibetan and Xingjiang breed (Liu YP et al., 2009) and other western region of Asia like in Pakistan (Sultana et al. (2003)). We speculate the strong gene flow among goat population was facilitated by the traditional seasonal pastoralism and annual long distance migrations in history as well as ancient trade from Tibet through Nepal to India would account for the pattern discerned in regional goat gene pool.

Conclusion

mtDNA control region (d-loop) sequencing HVI fragments were analyzed to test Nepalese goat phylogeny as well as to discern the genetic diversity of goat breeds/populations. In this study, we observed high mtDNA diversity in Nepalese goat breeds. The four mtDNA lineages A–D found in Nepalese goat breeds further support the previous view of multiple maternal origins of domestic goats (Joshi et al. (2004); Luikart et al. (2001); Sultana et al. (2003)). These results indicated that there was no correspondence between the geographic regions of origin and relationships among breeds. The study also shows the extensive gene-flow among goat breeds of different neighboring countries which throws the light the route of gene flow to Southern Asia from China considering as the origin of Haplogroup B (Sieh, 1985; Yu et al. (2009)) might be through Nepal. However, extensive study covering the goat breeds of the region and also using different markers suchas paternally derived Y chromosome and nuclear markers, considering the limitation of mitochondrial markers should be measured for the concrete conclusion.

Literature Cited

Neopane S.P., Gorkhali, N.A. and Pokhrel, P.K. (205). Published by Animal Breeding Division, NARC.