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Paternal Lineage Analysis in Sahiwal, Cholistaniand Dajal Cattle Breeds of Pakistan through *SRY* gene

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Abstract: This was the first study conducted to determine the origin and genetic diversity of Pakistani cattle (Cholistani, Sahiwal and Dajal). We analyzed *SRY* gene sequences of 60 samples, together with the available sequences in GenBank. The sequence of SRY gene is conserved in Pakistani cattle having no SNP. The neighbor joining tree showed that Pakistani cattle mostly originated from *Bosindicus*. To determine the percentage of genetic divergence for *SRY* gene in bovine and out-group, the p-distance model was used in the analysis. A very short genetic distance was found between our haplotypes (Sahiwal, Cholistani and Dajal) and *Bosindicus* (0.01%), showing a very high genetic similarity.

Key words: Pakistani Cattle · SRY Gene · Genetic Diversity · SNP

INTRODUCTION

Cattle play a vital role in the progression of human civilization. There are two main types of cattle which are *zebu* (Humped) and *taurine* (Without humps) and they are considered as a separate species called (*Bosindicus*and*Bostaurus*) due to complete inter fertility [1].

Cattle are an important member of subfamily Bovine and the most popular species of genus *Bos*. Cattle play an important role in livestock and can be used as meat animals, as dairy animals for milk and other dairy products and as draft animals. In addition to this other products such as leather and dung for energy (Fuel) are also obtained from cattle. In some countries, such as India, cattle are holy animal. It is predictable that there are 1.3 billion cattle present in the world today. Cattle population of Pakistan is about 29.6 million heads, in which 49% are present in Punjab, 23% in Sindh, 20% in Khyber Pakhtoonkhwa and 8% in Balochistan [2]. All the cattle of Pakistan belong to zebu (Humped type) cattle (*Bosindicus*). There are 15 breeds of cattle in Pakistan which are Cholistani, Sahiwal, Dajal, Dhanni, Red Sindhi, Tharparkar, Achai, Lohani, Bhagnari, Gabrali, TaurindicusRojhan, Kankraj, Hissar and Hariana. Out of these breeds Sahiwal, Cholistani, Red Sindhi and Tharparkar are well known as dairy cattle breeds internationally and all have been used for producing new breeds. The Lohani, Achai and Gabrali are small sized breeds and generally grouped as Desi, while Bhagnari, Dajal, Dhanni, Kankraj and Rojhan are known as Draft cattle breeds [3].

Among the cattle breed of Pakistan Sahiwal cattle are regarded as the best cattle breed for milk production and is an important genetic resource of Pakistan. The Cholistani breed is also one of the important cattle breed of Pakistan and known to be a multi-purpose breed, which can be used for both milk and meat and can also be used as a draft animal. Cholistani are usually black, brown or speckled red [4]. Dajal is a famous cattle breed of Pakistan and grouped in the draft cattle breed. The breed is found in the Dajal areas in district Dera Ghazi Khan of the Punjab province. The study of mitochondrial phylogenies is complex due to intra species hybridization

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whereas the deviation of nuclear gene sequences is not much enlightening at this level [5,6]. On the other hand Y-Chromosome deviation appears to be a reliable marker for bovine phylogeography and convergence [7,8]. In general, Y chromosome haplotyping is paired to data from markers based on mitochondrial DNA but the main difference is that mitochondrial DNA study is a maternal study while the Y chromosome study allows particularly paternal relationships to be studied. Due to Y chromosome haplotyping we are able to evaluate directly the relative role of fathers in shaping the genetic signatures of populations [9].

To delineate the actual origin of present Asian local cattle and to describe the degree of genetic introgression in Asian local cattle the sequences of the Cytochrome b gene, mtDNA and *SRY* gene (sex determination region of the Y chromosome) are studied [10]. *SRY* is conserved region and therefore no recombination occurs within it just like mtDNA, but the mtDNA is a female-specific molecular marker.

Hence the current study is planned to see the phylogenic analysis of major Pakistani cattle with the following objectives.

- To identify genetic difference of Pakistani cattle breeds through Single Nucleotide Polymorphism (SNPs) detection in Y-chromosomal *SRY* and *ZFY* genes.
- To study the evolutionary relationship of major cattle breeds in Pakistan.

MATERIALS AND METHODS

The work was carried out at Molecular Biology and Genomics Lab, Institute of Biochemistry and Biotechnology, University of Veterinary and Animal Sciences, Lahore. In this study, blood samples from 60 true representative animals of each of three cattle breeds (Sahiwal, Cholistani and Dajal) were collected from different Government and private livestock farms and their respective home tracts. Genomic DNA will be extracted from blood samples by inorganic method [11] which involves RBCs lyses, protein digestion and precipitation followed by DNA isolation and purification. The DNA samples will be dissolved in Tris EDTA (TE) buffer (pH 8.0) and stored at -20°C for further use. Two set of primers were designed from Sex Determining Region of Y Chromosome (SRY) of BosTauras Table 1 [12]. The size of SRY gene is 2831bp (GenBank Accession No. AB039748). Primerswere designed usingprimer3 software (http://frodo.wi.mit.edu/).

Table 1: Primer sequence designed from Sex Determining Region of Y Chromosome (SRY) of Bos Tauras

Serial No.	Primer Name	5'-3' Sequence
1	SRY1-F	TTGCACTAAGTCAGTCTGTGGTAA
	SRY1-R	TCGGGTTGCATAGTATTGAAGA
2	SRY2- F	ACTAGCCATACACCGAGACAAA
	SRY2- R	GGACCAGTTATATTGGAAAGTCTG

DNA was amplified from *SRY* in a total volume of 25 µL reaction mix, 2 µL genomic DNA, 2.5 µL dNTPs, 2.5 µL $MgCl_2$, 2 µL PCR buffer, 0.75 µL of each primer and 0.2 µL Taq DNA polymerase. PCR conditions for *SRY*gene primers were 94°C for 4 minutes, followed by 94°C for 30seconds, 52°C for 45 seconds and 72°C for 45 seconds for 30 cycles and a final step of 72°C for 10 minutes. Precipitation of the PCR product is done after the amplification of the desired portion of the DNA. After the precipitation, PCR products were sequenced. Sequencing is done on the principle of Sanger Chain Termination. The obtained sequences were aligned with the help of online software blast 2 sequences (www.ncbi.nlm.nih.com) with reference sequence of *SRY* from which primers were designed. Single nucleotide polymorphisms were calculated from the observed sequences of Y chromosomal *SRY*gene. From the calculated SNPs, haplotypes were identified. The Neighbor Joining Tree was constructed from these haplotypes with MEGA program package, MEGA 5 [13].

RESULTS AND DISCUSSIONS

Studies on Y chromosome are of particular interest in livestock species because in common breeding strategies only a few males contribute genetically to the next generation [14]. According to the previous reports *SRY* gene is highly conserved [15] and our results also shows that the SRY gene in cattle is highly conserved, having no polymorphic site in all the three breeds Sahiwal, Cholistani and Dajal.

A Neighbor Joining Tree was constructed using MEGA5 with SRY haplotypes of Cholistani, Sahiwal and Dajal and different species including *Bostaurus, Bosindicus*, *Bubalusbubalis, Synceruscaffer, Bosgrunniens* and *Capra hircus* (Fig. 1). Two different clades "A" and "B" are formed. The clade A consisted of two embranchments, one branch included *Bos Taurus* and the second branch is consisting of *Bosindicus*, *Bosgrunniens* and our haplotypes (Sahiwal, Cholistani and Dajal). The clade B consisted of two buffalo breeds *Bubalusbubalis* and *Synceruscaffer*.

Table 2: Percentage divergence for *SRY* Gene sequence

	Haplotype	Bosindicus	Bostaurus	Bubalusbubalis	Synceruscaffer
Bosindicus	0.010				
Bostaurus	0.140	0.144			
Bubalusbubalis	2.750	2.753	2.898		
Synceruscaffer	2.608	2.608	2.750	2.318	
Capra hircus	7.530	7.530	7.530	8.550	0.0841

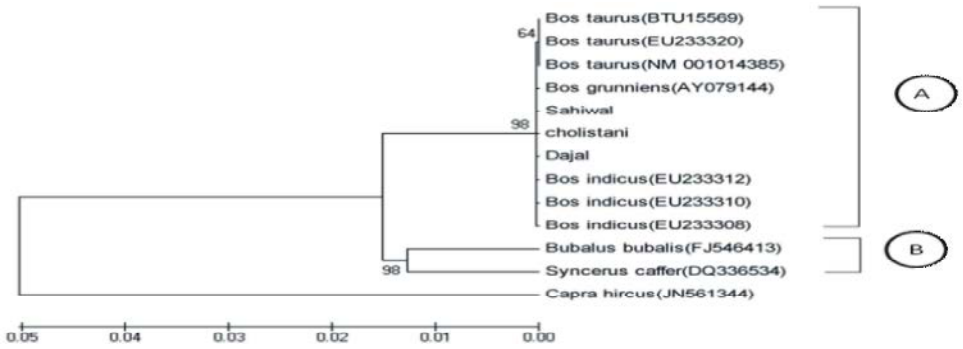

Fig. 1: A rectangular shape Neighbor Joining Phylogenetic Tree of *SRY* gene

These results indicate that Cholistani, Sahiwal and Dajal cattle breeds of Pakistan are *Bosindicus.* The haplotypes of these breeds based on *SRY* gene was closely related to each other. The close association and low levels of variation in haplotypes of these breeds indicated that all the three breeds belonged to same geographical regions [16].

To determine the percentage of genetic divergence for *SRY* gene in Bovinae and out-group, the p-distance model for detecting genetic distance was used in the analysis. A very short genetic distance was found between our haplotypes (Sahiwal, Cholistani and Dajal) and *Bosindicus* (0.01%), showing a very high genetic similarity. The percentage of nucleotide sequence divergence between our haplotypes (Sahiwal, Cholistani and Dajal) and *Bosindicus* (0.01%) was lower than that of our haplotypes (Sahiwal, Cholistani and Dajal) and *Bos Taurus* (0.14%). Meanwhile, genetic comparability of our haplotypes (Sahiwal, Cholistani and Dajal) with *Synceruscaffer* (2.6%) and *Bubalusbubalis* (2.75%) is much higher. The higher divergence in the nucleotide sequence is present between our haplotypes and *Capra hircus* (7.53%) which was taken as out-group in the phylogenetic tree (Table 2).

CONCLUSIONS

The present study might be helpful for the conservation of the Pakistani cattle breeds, as the conservation of local animal diversity is important to meet upcoming needs of the region. In order to manage with an unpredictable future, genetic reserves capable of readily responding to directional forces imposed by a broad spectrum of environments must be maintained. Maintaining genetic variation is an insurance package against future undesirable conditions. Due to variation among environments, dietary standards and challenges from infectious agents, a variety of breed and populations are required. These act as depot of genetic variation which form the basis for collection and may be drawn upon in times of biological stress such as food crisis, drought or disease epidemics. The wide range of breeds and species are each specially adapted to a different set of situation.

Competing Interest: The author declares that they have no competing interest.

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