Journal of Animal & Plant Sciences, 32(1): 2022, Page: 319-324
ISSN (print): 1018-7081; ISSN (online): 2309-8694
https://doi.org/10.36899/JAPS.2022.1.0427
Short Communication

PHYLOGENETIC STUDY OF SOME SAUDI GOAT BREEDS BY USING SEQUENCE ANALYSIS OF SPECIFIC MITOCHONDRIAL D-LOOP REGION

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ABSTRACT

Goats were raised in different regions of the world for various purposes including milk and meat production as well as skin and fiber. The number of goats evaluated to be about 2.5 million heads, which represent about 30% of total meat income of Saudi Arabia. Saudi local goat breeds did not receive the required attention about their genetic information, despite their important adaptive characteristics. This study aims to access genetic diversity of some Saudi goat breeds depending on sequence analysis of specific mitochondrial D-loop region. Results showed that the Saudi goats resemble two main groups (1 and 2). Group one, is classified into two subgroups. Subgroup 1a (Saudi goats with accession numbers MG725669, MG725670 and MG725673 of Habsi breed) and Subgroup 1b (Saudi goats with accession numbers MG725674, MG725676 and MG725677 of Harri breed). Lineage A (group one), showed characteristic difference compared to all other studied goat members around the world. The nearest goat to this Lineage is goat accession number (KJ940969). On the other hand, Saudi goats outside this group (group 2) showed high similarity with other sequences of goats obtained from GenBank database, which included in this study. The results of Lineage 2 (group 2) showed no noticed sharp difference with the published GenBank database sequences. Finally, Lineage 1(group one) showed novel results including specific SNPs and one deletion, which is characteristic only to this group. This will give more insights about correlation between sequence, classification and function of these breeds.

Key words: Mitochondrial D-loop, Phylogenetic study, Saudi goat breeds

Published first online June 14, 2021 Published final January 07, 2022.

INTRODUCTION

Goat are considered one of the earlier domesticated farm animals. The archaeological studies reveal that goats have been related with men in mutual relationships (Ensminger and Parker1986). Goats deployed overall world because of its ability to adapt to large different environmental conditions and different food systems, which evolved and maintained thereafter. In addition, they are helpful to humans because of their productivity, their small size and small competitiveness for food resources. In developing countries these animals have very important contributions especially in rural areas. However, there is underestimation of this genetic resource and their contributions to the poor livelihood. Goats are often ignored when compared with both cattle and sheep. The reason of that would be probably due to the recognition of their ability, rather than any prejudice against them, as goats have very distinctive characteristics such as tolerant to parasites and pathogens, intelligent and independent. In addition, they have the ability to take care of themselves along with their young one, compare to other animals (Abdel Aziz,2010).

Goats raised in different regions of the world for various purposes including milk and meat production as well as skin and fiber. Their number is evaluated to be about 2.5 million heads in Saudi Arabia and this would be due to suitable climatic circumstances for this animal species (Salah *et al*., 1989). Ardi, Masri and Habsi are most abundant breeds in Saudi Arabia (Al Saidy *et al*., 2007). Ardi goats characterized by high adaptation to arid climatic circumstances. Also, Ardi goat breed is favorite by local animal breeders for their steady milk production under harsh conditions in Saudi Arabia. Goat animals represent about 30% of total meat income of Saudi Arabia. In spite of important adaptive characteristics of these animals, they did not receive the required attention about their genetic information, which is necessary for enhancing the productive performance of such animals. Little studies are available regarding the phylogenetic relationship among Saudi goat breeds. In addition, few researches were conducted for genotyping of such important economic traits of Saudi goats. The limitation of such information leads to delay for the improvement of these local animals in the field of genetic engineering. There is very little valuable genetic information available among and within goat species in kingdom of Saudi Arabia. In this context, Sabir *et al* (2012) conducted a study using RAPD technique to access genetic similarities among four Saudi goat breeds. The results of this study indicated that Ardi and harri breeds have higher genetic similarities 73.5%. Also, these breeds share about 65% genetic similarity with that of Masri goat and Habsigoat breeds respectively. In addition, Sabir *et al.,* (2013) used Inter-Simple Sequence Repeats (ISSR) to access genetic variation between three Saudi goat breeds. The results indicated that Harri and Aradi goat breeds grouped together where Najdi breed was group apart. In addition, El Hanafy *et al.,* (2015,2016)studied the relationship between milk production trait and polymorphisms of β-Lactoglobulin and α-lactalbumin genes in native Saudi breeds. This latest information would be of the greatest important in term of enhancing the productive performance of these animals.

Different molecular methods were developed for exploring phylogenetic relationships, relatedness between farm animals' species as well as parentage analysis (Feral, 2012).In recent years, exploring whole genomes considered to be one of the important comparative and function identification studies. However, these studies are not easy to perform and more expensive. Polymerase chain reaction (PCR) represents breakthrough in methodology (Mulis& Faloona,1987; Sakai *et al*,1988). This method has been widely used for genetic variations among and within species of different animal's studies. This technique used in the represent method for amplifying specific sequence in vitro. In this context, Sequence of specific mitochondrial 16S rRNA gene fragment used as a pattern for discrimination between closely related species including Goats (Ramadan, 2011).

The D-loop is a non-coding, most variable but functionally important region of the mammalian mtDNA genome. This area is useful for the studies of phylogenetic analysis as well as for genetic variability among populations.(Upholt & David ,1977; Simonsen *et al*,1998;Kierstein *et al* ,2004).Recent studies based on mtDNA sequences have been designed to illustrate the origin and genetic diversity of Caprine breeds (Luikart *et al*.,2011; Sultana *et al.,*2003;Joshi *et al.,*2004).These studies have permitted identification of several maternal lineages, hence demonstrated that the goat domesticated from different populations of the wild bezoar goat (*Capra aegagrus*). Five maternal lineages (A–E) described for domestic goats based on mtDNA sequence data.

The mtDNA is unique in its properties. These properties could be attributed to maternal inheritance, higher substitution rate, and very lower recombination rate (Ganbold *et al*., 2019;Abd-Elhakim*et al*.,2020). The control region, also called displacement-loop (D-loop) region, is the primary noncoding controlling area for mtDNA replication and transcription. The structure and variation of the mtDNA control region give it advantages to designate the genetic polymorphisms and maternal source, chiefly since mtDNA shows slight maternal inheritance without reconstruction and with a reasonably fast evolution rate (Hiendleder *et al*., 1998; Abd-Elhakim*et al*.,2020).

Polymorphisms of mitochondrial DNA (mtDNA) are widely used to detect the origin, ant study the processes of domestication, migration routes of domestic goats and their wild relatives, as well as to establish phylogenetic relationships via maternal lineages This can be achieved via sequencing of different regions of mtDNA, including the hypervariable region HV1. D-loop, cytochrome B, protein coding genes and the complete mitogenomes (Deniskova *et al*.,2020).

Information generated from genetic diversity among domestic animals represents important tool. This helps us in the improvement and adaptation with different environment. (El-Hanafy&El-Saadani,2009; El-Hanafy *et al.,*2010). Few studies are available about the phylogenetic analysis of goat species in the kingdom of Saudi Arabia. The aim of the study was to explore the genetic diversity among and within the species. This information can be used for the improvement of local Saudi breeds to adapt harsh environment.

MATERIALS AND METHODS

Experimental animals: Blood samples were collected from different farms in Jeddah, Al-Qunfudhah village and Riyadh kingdom of Saudi Arabia. Habsi, Harri and Ardi were the three breeds selected for this study. All experimental procedures were approved by the Ethics Committee King Abdulaziz University with Reference No. 298-14 Animal study, 10 November 2014.

PCR amplification of the mtDNA: Total Genomic DNA from all the blood samples were extracted using blood DNA QIAamp DNA extraction kit according to manufacture procedure. PCR amplification of specific fragment of the mtDNA control region (800-bp) was done using primers CGCTCGCCTACACACAAATA and AATGCCCATGCCTACCATTA (Amills *et al,*2004).

Sequence analysis and multiple sequence alignment: Selected fragments sequenced on both strands following the Sanger's dideoxy chain termination method (Sanger *et al.*, 1977).For each animal forward and reverse specific D-loop Sequences were corrected using BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/) to have final forward corrected sequence. These obtained sequences submitted to GenBank database and got these accession numbers (MG725664-MG725678).

BLASTN version 2.2.5 and PSI BLAST was used for the Pairwise sequence alignments (Altschul *et al*..1997). MUSCLE 3.6 software and CLUSTALW 1.82 were used for the multiple sequence alignments and variable sites extraction of D-loop sequences of goat species (Edgar, 2004; Thompson *et al.*, 1994).

RESULT AND DISCUSSION

PCR amplification of specific fragment of the mtDNA control region (800-bp): Figure 1 represents PCR amplification of specific fragment of the mtDNA control region (800-bp) of different Saudi goat breeds.

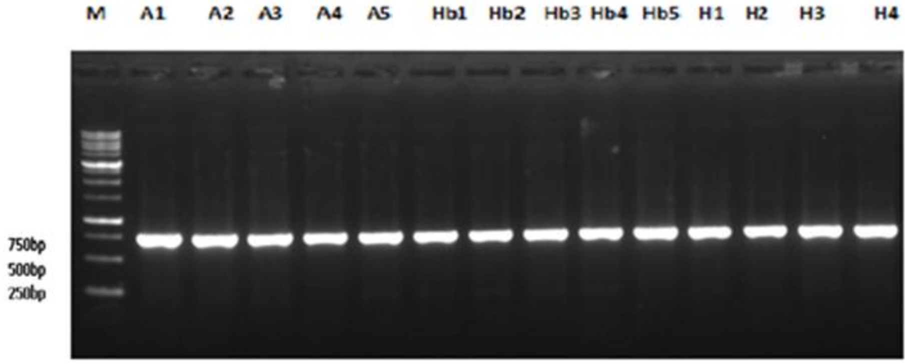
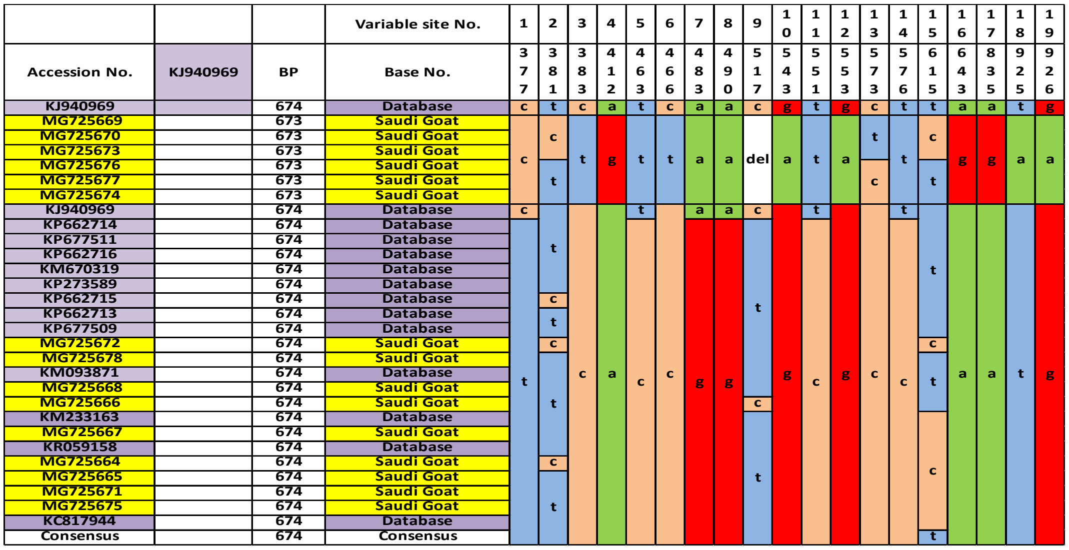


Figure (1) PCR amplification of control region of the mtDNA (800-bp) for different Saudi goat breeds. (M) 1kbp DNA ladder, (A) Ardi, (Hb) Habsi and (H) Harri.

Sequence analysis and alignment with gene bank data: Sequence analysis represented in figure 2. This figure illustrated nucleotides variation between Saudi goats used in the current study and sequence data obtained from gene bank database.

Results showed that the Saudi goats classified into two main groups. Group one classified into two subgroups. Subgroup 1a (Saudi goats accession numbers MG725669, MG725670 and MG725673 of Habsi breed) and Subgroup 1b (Saudi goats accession numbers MG725674, MG725676 and MG725677 of Harri breed). Group 1 showed characteristic difference compared to all other goat members. The nearest goat to this group is accession number obtained from GenBank database (KJ940969). We used this accession number as a reference to identify the variation in nucleotides between all chosen goats.

Goats of Group 1 plus goat accession number (KJ940969) as one group were completely more different from other goats in nucleotides composition. They showed nucleotide cytosine at position 377 where all other goats showed thymine. They showed nucleotide thymine at position 463 where all other goats showed cytosine. At positions 483 and 490 they showed nucleotide adenine where all other goats showed guanine. They showed nucleotide thymine at positions 551 and 576 where all other goats showed cytosine.



			Variable site No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Accession No.	KJ940969	BP	Base No.	377	381	383	412	463	466	483	490	517	543	551	553	573	576	615	643	835	925	926
KJ940969		674	Database	c	t	c	a	t	c	a	a	c	g	t	g	c	t	t	a	a	t	g
MG725669		673	Saudi Goat	c	c	t	g	t	t	a	a	del	a	t	a	t	t	c	g	g	a	a
MG725670		673	Saudi Goat																			
MG725673		673	Saudi Goat																			
MG725676		673	Saudi Goat		t											c		t				
MG725677		673	Saudi Goat																			
MG725674		673	Saudi Goat																			
KJ940969		674	Database	c	t	c	a	t	c	a	a	c	g	t	g	c	t	t	a	a	t	g
KP662714		674	Database	t				c		g	g	t		c			c					
KP677511		674	Database																			
KP662716		674	Database																			
KM670319		674	Database																			
KP273589		674	Database																			
KP662715		674	Database		c																	
KP662713		674	Database		t																	
KP677509		674	Database																			
MG725672		674	Saudi Goat		c													c				
MG725678		674	Saudi Goat		t													t				
KM093871		674	Database																			
MG725668		674	Saudi Goat																			
MG725666		674	Saudi Goat									c										
KM233163		674	Database									t						c				
MG725667		674	Saudi Goat																			
KR059158		674	Database																			
MG725664		674	Saudi Goat		c																	
MG725665		674	Saudi Goat		t																	
MG725671		674	Saudi Goat																			
MG725675		674	Saudi Goat																			
KC817944		674	Database																			
Consensus		674	Consensus															t				

Figure (2) variable sites of the D loop sequences between all studied goats related to goat accession number KJ940969.

Goats of Group 1 only showed very specific nucleotides, which characterized this group. They showed nucleotide thymine at position 383 and 466 where all other goats showed cytosine. They showed nucleotide adenine at position 543, 553 and 926 where all other goats showed guanine. They showed nucleotide guanine at positions 643 and 835 where all other goats showed adenine. They showed nucleotide adenine at position 925 where all other goats showed thymine. The most important characteristic of this group is identification of a deletion at position 517, which was not indicated in other goats. Most goats have thymine nucleotide and less have cytosine nucleotide at this position.

In order to differentiate between subgroup 1a (Habsi breed) and subgroup 1b (Harri breed) : subgroup 1a showed nucleotide cytosine at positions 381 and 615 while subgroup 1b showed nucleotide thymine. Subgroup 1a showed nucleotide thymine at positions 573 while subgroup 1b showed nucleotide cytosine.

On the other hand, Saudi goats outside this group (group 2) showed high similarity with other goats included in this study with no noticed sharp difference between them. The neighbor joining tree (Fig 3) show the cluster of group one with goat accession number (KJ940969) forming a separate group compared to other goats.

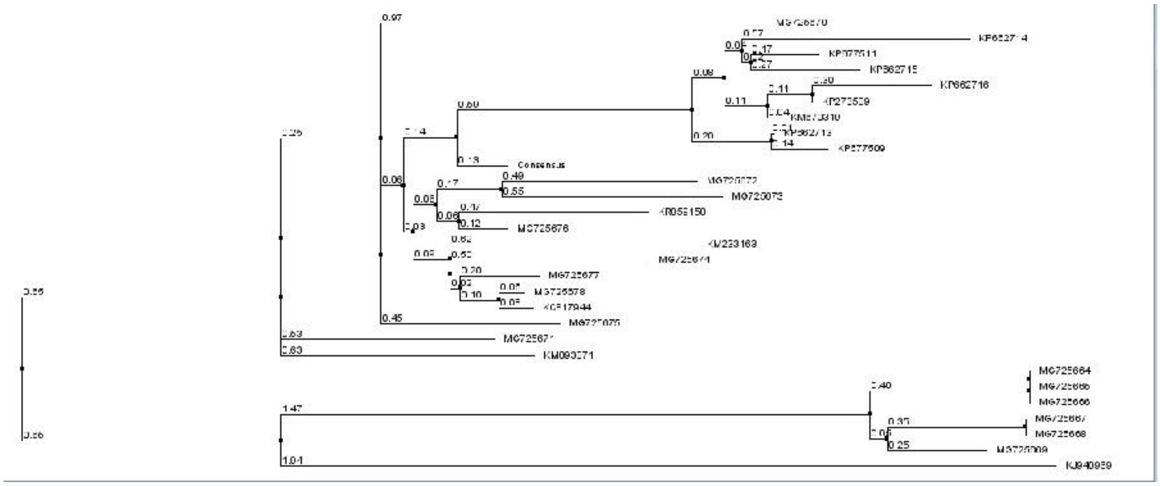


Figure 3: Neighbor-joining tree for all studied Saudi goats compared to GenBank database goat samples.

It can be concluded that Harri and Habsi goat breeds have higher genetic similarities based on phylogenetic analysis while Ardi breeds lay away from these two previous breeds. These results disagree with the previous results obtained (Sabir *et al*.,2012) in which RAPD technique was used to access genetic similarities among four Saudi goat breeds. They indicated that Ardi and Harri breeds have higher genetic similarities 73.5%. Also, these breeds share about 65% genetic similarity with that of Masri goat and Habsi goat breeds respectively. In addition, Sabir *et al* (2013) used Inter-Simple Sequence Repeats (ISSR) and indicted that Harri and Aradi goat breeds grouped to gather where Najdi breed was group apart. This disagreement with the previous results could be explained on the basis that sequence alignments of D-loop is more accurate in accessing genetic relationships between caprine breeds than both RAPD and ISSR techniques. The D-loop is a non-coding, most variable but functionally important region of the mammalian mtDNA genome. This area is useful for the studies of phylogenetic analysis as well as for genetic variability among populations. (Upholt& David ,1977; Simonsen *et al*,1998; Kierstein *et al* ,2004).

In agreement of our work Deniskova*et al* (2020) used D-loop mitochondrial DNA (mtDNA) sequences715 bp to access maternal origin of seven Russian local goats' populations. Their results indicated total of 59 haplotypes were determined in the Russian goat breeds, in which all differed from the haplotypes of the Saanen goats. The haplotypes identified in Altai Mountain, Orenburg, Soviet Mohair, and Saanen goats were breed specific. and they reveal a high haplotype (HD = 0.843–1.000) and nucleotide diversity (π = 0.0112–0.0261).in these breeds.

In another study Lestaria *et al* (2018) relationships between Kejobong goat and Kacang goat as well as Etawah Grade goat was investigated using genetic diversity, haplotype, and genetic distance analysis based on D-loop sequences. They concluded that genetically, Kejobong goat is a crossbred of Kacang goat and Etawah Grade goat and has closer genetic relationships to Kacang goat than to Etawah Grade goat.

Acknowledgments: This work was supported by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, under grant No. (130-58-D1436) The authors, therefore gratefully acknowledge the DSR technical and financial support.

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