Academic Journal of Nawroz University (AJNU), Vol.11, No.4, 2022

https://doi.org/10.25007/ajnu.v11n4a1463

Mitochondrial DNA Analysis of Awassi-Iq (Ovies aries) Sheep Breeds from Nineveh Province, Iraq

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ABSTRACT

In Iraq, Awassi is the most widespread sheep breed of non-European origin. The breed is adapted to a wide range of environmental conditions. Sheep farming, mainly sheep of Awassi breed, represents an essential economic activity of Iraq; however, so far, only very few genetic data exist on this breed. The present study was performed in Biology Department, University of Zakho. A total of 32 blood samples were obtained from Awassi-Iq (Ovis aries) sheep breed in three farms located in Nineveh province, Iraq. Genomic DNA extraction was performed using spin column method. Specific marker (GenBank: NC001942) used for amplifying 550 bps fragments of mitochondrial DNA in PCR assay. The PCR products were separated by electrophoresis on 1.5% agarose gel, visualized by staining with RedSafe dye, and photographed by a digital camera. Ten PCR products were sequenced and compared to sequences from other countries available in NCB1.The main results indicate that Awassi- 1q (Ovis aries) sheep breed shares similarity to both Chines Tibetan sheep breed (Linzhou); Iranian sheep breed (91.37%), to both Iraqi sheep breeds Hamdani; Nima (91.62%) respectively and to Ovis aries AKK- 46 from Turkey (92.35%). However, Awassi-Iq was shown to be monophyletic, out group breed and diverged earlier than related breeds (Turkey, Iran, and China). This study highlights the importance of Mitochondrial DNA analysis in genetic study of sheep breeds.

KEY WORDS: Awassi-Iq sheep breed, Ovis aries, mitochondrial DNA, and Nineveh.

1. Introduction

Sheep are known to be one of the first domesticated animals, since the history of domesticated sheep began in the Fertile Crescent around 11,000 BP (Zeder, 2008). In Iraq, sheep breeding is an important livestock sector, which contributes significantly to the food security and welfare of rural households. The sheep population of Iraq in 1999 was about six-million head (FAO, 2000). Most of this population (99.8%) is owned by the private sector (Ministry of Planning, 1987). In Iraq, Awassi sheep (*Ovis aries*) is the dominant fat tail breed, that has many unique characteristics, including acceptable performance under harsh conditions and preferred meat quality by consumers. They are carpet-wool producers with some potential to produce milk. Most of the income of sheep producers is derived from the sale of lambs. Milk is partially consumed by the farmers as an important source of protein and the remainder is sold as ghee, yogurt and cheese (Iñiguez, 2005; Al-Dabbagh, 2019).

For Iraqi sheep breeds, earlier studies based on morphological and biochemical markers did not present a true picture of their relationships. These markers have many limitations in identifying breed specificity as they do not have high resolving and distinguishing power among closely related breeds in term of coat color, horn types, limited polymorphic nature of the serum protein or biochemical variants (Meadows *et al*., 2006).

In recent years several genetic markers such as mtDNA, Y-chromosome markers, autosomal microsatellites and whole-genome SNPs are widely used in domestic animal studies (Meadows *et al*., 2006; Chessa *et al*., 2009; Wallner *et al*., 2013). One of effective methods of studying the genetic of animals is the analysis of the mitochondrial DNA (Tapio *et al*., 2006). Several studies have been conducted in Iraq to estimate the genetic diversity or polymorphism inside and among native sheep breeds using molecular markers (Mohammed, 2009; Mohammed and Jubrael, 2010; El Hage, 2017). To the best of our knowledge mitochondrial DNA analysis in sheep breeds has not been reported in Nineveh province, Iraq. Therefore,

this research aimed to analyze the mitochondrial DNA from Awassi-Iq sheep breed followed by the comparison of the genetic relationships with other breeds from countries worldwide using the mitochondrial DNA control region sequence.

2. Materials and Methods

From March to April 2021 blood samples were obtained from 32 individuals of Awassi-Iq (*Ovis aries*) sheep breed from three farms located in the Zummar area (North West of Nineveh province); Rabea'a (West of Nineveh), Tal afar area (West of Nineveh). Five ml of Blood from each individual were collected from the jugular vein into Ethylenediamine Tetra-Acetic Acid (EDTA) -containing tubes and stored in the freezer (-18 ˚C). The genomic DNA was extracted from blood samples using spin column commercial kit provided by Jena Bioscience GmbH (Germany) based on the manufacturer instruction supplied with the kit. Following genomic DNA extraction, the Nanodrop Spectrophotometer (Thermo Scientific, US) was used for determination the concentration and purity of the extracted DNA.

Polymerase chain reaction (PCR) was performed using a pair of species-specific primer were used; their sequences were obtained from NCBI data base (GenBank: NC001942). The primer was with following sequence:

(F: 5'GCCCCACTATCCAACACCCAAAG'3).

(R: 5'AATGGGCGATTTTAGATGAGATGGC'3).

The amplification reaction was performed in a 40 µl reaction tube consisting of 20 µl Master mix 2x, 4 µl of (10 pmol/ µl) primer (2 µl of forward and 2 µl of reverse), 4 µl of DNA template and 8 µl of deionized distilled water. The amplification was performed using thermocycler (Gene AMP PCR system 9700 Thermocycler). The PCR cycling protocol was as follows; one cycle of initial denaturation at 95 ˚C for 5 minutes; then 30 cycles of denaturation at 95˚C for 20 seconds, annealing at 59˚C for 25 seconds, and extension at 72˚C for 1.5 minutes; and followed by one cycle of final extension at 72˚C for 5 minutes.

PCR products were run on 1.5% (w/v) agarose dissolved in 100 ml of 1X TBE buffer and stained with RedSafe Dye. The gel was run for 15 min at 45 Volt then raised to 85 Volt for 60 min. A DNA ladder of 100-1000 bps was added as references ladder for determining the band size. The gel was viewed using UV Transilluminator to confirm amplification (Mohammed *et al.,* 2022).

Ten amplicons of sheep breed were chosen based on different locations were submitted to Macrogen Company (Korea) for DNA sequencing. The BLAST Software (Basic Local Alignment Search Tool) was applied for sequence analysis database to identify the obtained DNA sequences. All sequences were trimmed and aligned by BioEdite application. For the construction of the phylogenic tree MEGA Software Neighbor Joining method and Kimura's 2-parameter model were used for analysis (Kumar *et al*., 2018).

3. Results

In this study, the highest concentration of the amplicons was 295 ng/µl; whereas the lowest concentration was 13 ng/µl. While the purity ranged from 1.7 to 2.0 checked at A260/A280 nm. The amplified Mitochondrial DNA of Awassi –Iq sheep breed produced DNA fragments of 550 bps (Figure, 1).

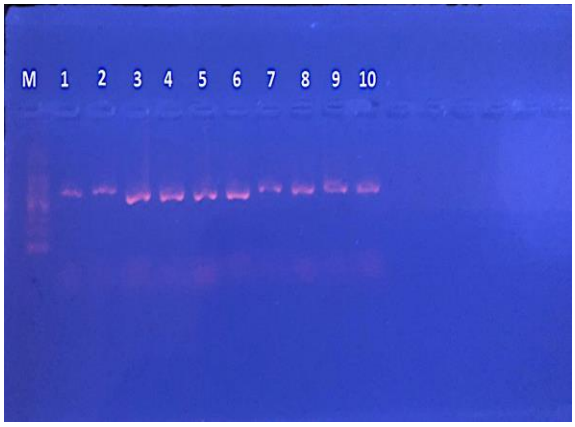

Figure (1): Agarose gel (size 550 bp) of PCR products of Awassi -Iq mtDNA obtained with species-specific primer. M: Ladder (100-1000 bp); 1 to 10: sample's number.

The obtained sequences were compared to the related sequences of *Ovis aries* sheep breeds. The Blast analysis of mtDNA showed similarity to references obtained

from GenBank (Table 1), and shared 91.37% similarity to both Chines Tibetan Linzho sheep breed (MK381462.1); Iranian sheep breed (MT768125.1) respectively, 91.62% to both Iraqi sheep breeds Hamdani and Nima (MF004242.1) and (LC649167.1) respectively and 92.35% to *Ovis aries* AKK- 46 from Turkey (KF677058.1).

Table 1: Accession numbers of mitochondrial DNA sequences obtained from Awassi-Iq sheep breed compared to references from different countries.

No.	Sheep breed	Accession Number	Countries	Similarity %
1	*Ovis aries* isolate HamdaniJ2	MF004242.1	Iraq	91.62%
2	*Ovis aries* isolate J49	KX344368.1	China	92.61%
3	*Ovis aries* Nima1	LC649167.1	Basrah, Iraq	91.62%
4	*Ovis aries* isolate T95	MK174516.1	China	92.35%
5	*Ovis aries* isolate Z49	KX344417.1	China	92.35%
6	*Ovis aries* isolate J50	KX344369.1	China	92.35%
7	*Ovis aries* isolate J1	KX344325.1	China	92.35%
8	*Ovis aries* isolate H45	KX344224.1	China	92.35%
9	*Ovis aries* isolate A3	KX344135.1	China	92.35%
10	*Ovis aries* isolate QH20	KP228453.1	Tibet, China	92.35%
11	*Ovis aries* breed Linzhou	MK381462.1	Tibet, China	91.37%
12	*Ovis aries* isolate AKK_46	KF677058.1	Turkey	92.35%
13	*Ovis aries* isolate SSS37	MT768206.1	China	91.37%
14	*Ovis aries* isolate Iran104	MT768125.1	Iran	91.37%
15	*Ovis aries* isolate DLS313	MT768110.1	China	91.37%
16	*Ovis aries* isolate BA60	MT768092.1	China	91.37%
17	*Ovis aries* isolate TBS87	EF494838.1	China	92.35%
18	*Ovis aries* breed Gangba	MK381455	Tibet, China	91.35%
19	*Ovis aries* isolate TJ12	KP228604.1	Tibet, China	92.35%
20	*Ovis aries* isolate GN36	KP228251.1	Tibet, China	92.35%

The phylogenic tree was constructed by comparing the obrained sequences with their accession numbers with international references of GenBank sequences of *Ovis aries* sheep breeds (Figure, 2).

Figure 2: Mitochondrial DNA likelihood phylogenetic tree of Awassi-Iq (*Ovis aries*) along with other breeds. Awassi-IQ (*Ovis aries*) was used as out- group. Major clades were assigned to corresponding haplotypes (A, B and C) based on Meadows *et al* (2011) classification

4. Discussion

Sheep domestication represents a milestone in the history of mankind. Sheep was one of the first animals to be domesticated in the Fertile Crescent. These domestication events, probably originated in the early Neolithic age, do not have genetically built the contemporary races of the Middle East but also of the whole world. Awassi is the most common breed of sheep in the east of Mediterranean. It is the main sheep breed in Iraq and Syria and the only native breed in Jordan (Hailat, 2005). Furthermore, it represents an importance contribution to sheep breed in Turkey (Gursoy, 2005) and Awassi sheep farming represents an essential economic activity of Iraq (Al-Dabbagh, 2019); however, so far, only very few genetic data exist on this breed. Nowadays, the molecular tools available allow us to define in details the genetic diversity of sheep populations and to trace their evolutionary history. This study provides the first phylogenetic analysis based on mitochondrial DNA in Iraqi Awassi sheep. Iraqi Awassi sheep breeds were found to share a genetic background and displayed genetic relationships with other native sheep breeds in Iraq, such as Hamdani and Nima breeds as they shared 91.62% similarity, respectively, Iranian sheep breed (Similarity 91.37%), and *Ovis aries* AKK- 46 from Turkey (92.35%). These findings are consistent with those reports on the history of domestication of sheep which initiated in the Fertile Crescent around 11,000 BP (Zeder, 2008). Subsequently, sheep were introduced to Eurasia through several routes that bypass Caspian Sea around 5,000 – 7,000 years ago (Tapio *et al.*, 2006). Several study on genetic characterization of the Awassi sheep breed using mitochondrial DNA markers have been reported in neighboring countries such as Lebanon and Jordan (El Hage 2017; Brake *et al*., 2021). The phylogenetic tree showed that the available breed's mitochondrial sequences are clustered in three main clades A, B and C (Figure, 2) based on *Ovis aries* haplogroup classification (Meadows *et al*. 2011). However, in the

present study, Awassi-Iq was monophyletic, out group breed and diverged earlier than related breeds (Turkey, Iran, and China) with a common ancestor in haplogroup C (El Hage, 2017). This agrees with previous studies suggesting that sheep were first domesticated in the Fertile Crescent region including Iraq and spread out worldwide (Chessa *et al*. 2009; Haddad *et al*., 2020).

5. Conclusion

The mitochondrial DNA of Awassi – Iq (*Ovis aries*) sheep breed was analyzed. The obtained results shared similarity to Chines Tibetan Linzhou sheep breed, Iranian sheep breed (91.37%), to both Iraqi sheep breeds Hamdani and Nima (91.62%), respectively and to *Ovis aries* AKK- 46 from Turkey (92.35%).

6. References

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