# CHARACTERIZATION OF TWO DOMESTIC GOAT BREEDS IN DUHOK PROVINCE / IRAQ USING MICROSATELLITES

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## Abstract

**In this study, a molecular– based technique was employed to characterize two Iraqi goat breeds. For this, thirty blood samples were obtained from two Iraqi indigenous goat breeds (Native and Merzi goat) in Duhok province. Genomic DNA was extracted, out of 8 microsatellites, 6 microsatellites were amplified and produced bands in polymerase chain reactions (PCR). PCR products were subjected to 1.5% agarose gel electrophoresis for fast and easy detection of successful amplification. Then, the PCR products were subjected to 6% Polyacrylamide electrophoresis and stained with silver nitrate. Many distinct alleles were observed that they can be indicative of possible breed marker.Close genetic distance was observed between Native and Merzigoat (D=0.1667). These results can be used to establish national conservation and breed improvement strategies.**

***Keywords****: characterization, Duhok, microsatellites,Merzi and Native goats.*

## 1. Introduction

In Iraq, domestic goat (*Capra hircus*)is an important livestock species, primarily raised for meat and milk (FAO, 2000). Goats contribute to 19.9% of the national income from ruminant and animal production. Furthermore, meat and milk from goats comprise 33.8% of the income of meat and milk of ruminants (Ministry of Planning, 1991). Iraqi indigenous goat breeds consist of Native and Merzi goats. The Native goats with a population of 1.6 million heads (FAO, 2000), is the only goat breed characterized in Iraq. The breed is raised primarily for meat and milk with hair of secondary importance (Mason, 1981).

Table 1: Information available on the morphological traits of native and Merzi goats.

| Traits | Native goat(Taha,1990) | Merzi goat(Masom,1981) |
|---|---|---|
| Coat color | Gray or black and occasionally pied or all white | White, red or brown |
| Body size | Medium | Small |
| Hair status | Coarse | Fine |
| Fiber length(cm) | 10-15 | 8-12 |
| Facial profile | Roman but slightly concave | Straight or slightly concave |
| Ears | Long and pendulous | Long and directed laterally |
| Horns | Males have long horns and female are polled | The horn of males reach 50 cm in length and female are polled |
| Body length(cm) | 65-70 | 50-55 |
| Body weight(kg) | 39-49.7 | 35-40 |

The hair is mainly used for manufacturing Bedouin tents and mats (Iniguez, 2005). The color of the coat varies with a dominance of gray or black and very occasionally pied or all white. White spots are found on the ears, head and/or legs. This goat is of medium size and suited to grazing over vast areas.Hair is coarse and of medium length (10-15 cm). Males usually have long horns while most females are polled (Table 1). The ears are long and pendulous. The head is narrow and facial profile is slightly concave. Males usually have medium to short beards.The average body weight of an adult male is 49.7 kg and of an adult female is 39 kg(Taha, 1990).

The Merzi goat is raised primarily for its fine hair. It is smaller in size than the Native goat

(Table 1). It is white, red or brown and some are a mixture of these colors. It is found in the northern region (Kurdistan) along the Iraqi northern border (Iniguez, 2005). According to Mason (1981) the Iraqi Merzi breed is similar, if not identical, to the Iranian Morghose (Pashmina or Cashmere). The facial profile is straight or slightly concave. The ears are directed laterally with a forward and downward inclination, and are 14-15 cm long. The horns of males reach 50 cm in length; they are twisted and vary from an open corkscrew to a tight vertical screw. Fleece is 25-30 cm long, usually yellowish white with a brown head, ears, neck, shoulders, and shank.

The characterization data of Local goats is imprecise; even some of them are uncharacterized such as Merzi goat (Iniguez, 2005) because 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 the closely related breeds in terms of coat color, horn types, limited polymorphic nature of the serum proteins or biochemical variants etc. Molecular markers are more accurate and reliable than all other markers because of their dense distribution in the genome, great variation, codominant inheritance and easy genotyping at DNA level (Koreth*et al*., 1996). Among the various molecular genetic markers such as Restriction Fragment Length Polymorphisms (RFLPs), Random Amplified Polymorphic DNA (RAPD) and Variable Number of Tandem Repeats (VNTRs), microsatellites (SSR) were found to be common in all eukaryotic genomes with frequencies as high as one marker per every 6 kband easy to type via polymerase chain reaction (Bechman and Weber, 1992).Microsatellites are co-dominant markers, so that all alleles can be scored. The availability of microsatellites markers has facilitated genetic linkage studies; including mapping and searching for genes affecting productive traits as well as estimating genetic diversity in farm animals (Jouquand*et al*.,2000; Moioli*et al*., 2001; Kumar *et al*.,2006). Most of the studies using microsatellites have concentrated on cattle, sheep and pigs, while information available about the genetic characterization of goats is limited (Barker *et al*., 2001).

This study aim was elucidating the genetic relationship between Native and Merzi goats using microsatellites.

## 2. Material and Methods

### 2.1. Samples

A total of 30 blood samples was collected during March to April 2010 from two domestic goat breeds found in Duhok province, 15 samples from each breed. Samples were collected in two farms located in the Sharya and Semmel. Sampling of pure breeds was considered. Bulked segregant analysis (BSA) has been used as a rapid procedure for identifying markers in specific regions of the genome (Michelmore*et al*., 1991).

Table 2.Animal's microsatellites

| Primers | Sequence | Location(chromosome) |
|---|---|---|
| OAR119 | CTC AGC AAA TGG TTC CTG GGC ACC<br>TTT TAT AGT GAG GTG ACC ACT TGA TG | 19 |
| EP7 | GAT CTG AAA CGT GAA GGG TG<br>GCA CTC TAG TAT TCT TGC CA | 2 |
| EP12 | GCA GAG TAA TCA GAG CTG C<br>CTA AGT AAG ACC TGG CTC CT | 14 |
| MC47 | CAA TAT CTT GTC CCA TCC CTG TTC<br>AGA TTT GGG TCA TCA GCT CTA TCA AG | x |
| MC58 | CTG GGT CTG TAT AAG CAC GTC TCC<br>CAG AAC AAT AAA CGC TAA ACC AGA GC | 1 |
| OAR266 | GGC TTT TCC ACT AGC TTT ACA TAG GAG TG<br>CAC CAC ATA CCA AAC ACA CAG CCT GC | 25 |
| MCM0042 | CAT CTT TCA AAA GAA CTC CGA AAG TG<br>CTT GGA ATC CTT CCT AAC TTT CGG | 9 |
| MCM0064 | TAC AGT CCA TGG GGT CAC AAG AG<br>TAC AGT CCA TGG GGT CAC AAG AG | 2 |

### 2.2. DNA Extraction and Genotyping

Genomic DNA was extracted using a method based on Proteinase K digestion and Phenol/Chloroform extraction (Ausubel*et al*., 1989). The Microsatellite genotyping technique was adopted in the study. The microsatellite loci were OAR119, EP7, EP12, MC47, MC58 and OAR266. The International Center for Agriculture Research in the Dry Areas (ICARDA) supplied all loci used in the study(Table2).

The PCR was performed in 20 µlreaction mixture consisting of approximately 100 ng of DNA, dNTPs (2 µl), PCR buffer (2 µl), forward and reverse primers (1 µl) for each one, *Taq polymerase* (0.2 µl ) and deionized distilled water (9.8 µl).The reaction mixture was overlaid with sterile mineral oil and was run in thermocycler. Touchdown PCR Protocol was performed by 1 cycle (initial denaturation) of 5 min at 94 □□ C, followed by 10 cycles of 30 sec of denaturation at 94 □□ C, 30 sec of annealing at 68 □□ C to 58 □□ C, and 30 sec of extension at 72 □□ C. 35 cycles of 30 sec of denaturation at 94 □□ C, 30 sec of annealing at 58 □□ C, and 30 sec of extension at 72 □□ C. 1 cycle of 5 min at 72 □□ C(final extension) .The amplified products were run firstly on 1.5% agarose gel for fast and easy detection of successful amplifications. Then PCR products electrophoresed in 6% 0.60 mm thick Polyacrylamide gel and the DNA bands were visualized by silver staining (Sanguinetti *et al.*,1994). The resulting microsatellite data were analysed using the Power Marker V3.25 Software. Genetic distance was computed using Nei's (1972) standard genetic distance (Ds) and the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method was used for the construction of phylogenetic tree.

## 3. Results and Discussion

The PCR products were analyzed on agarose gel electrophoresis to check for successful amplification.PCR reactions were then successfully obtained in repeated experiments with all primers except for MCM0042 and MCM0064.The results obtained in these experiments revealed that 6 out of 8 primers have amplified the specific regions and produced specific bands (microsatellite primers wereOAR119, EP7, EP12, MC47, MC58 and OAR266.

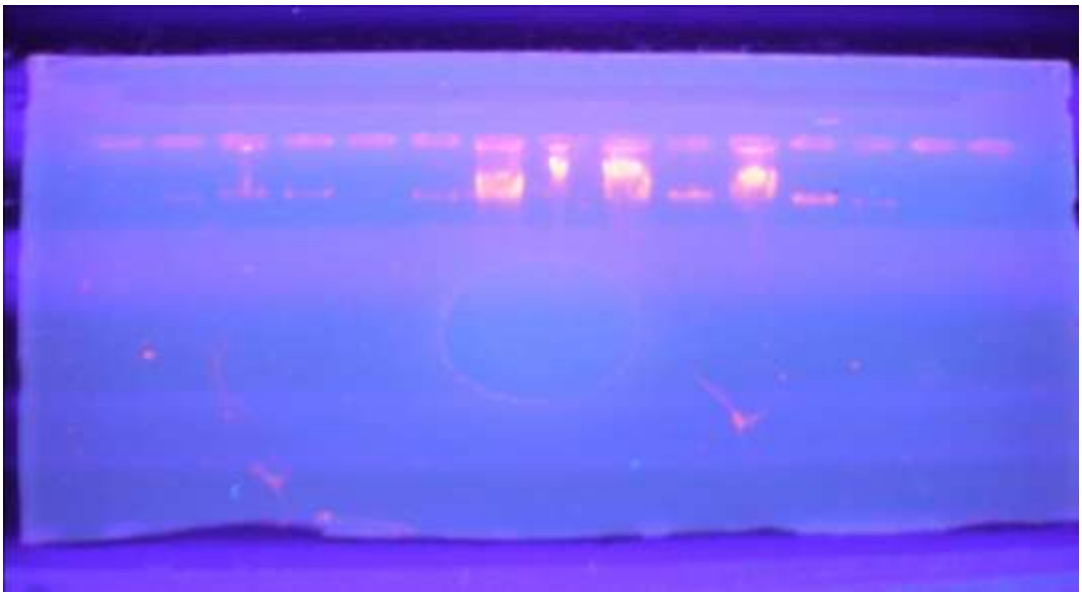

Figure 1.Amplified PCR products obtained with microsatellite primers on 1.5% agarosegel .

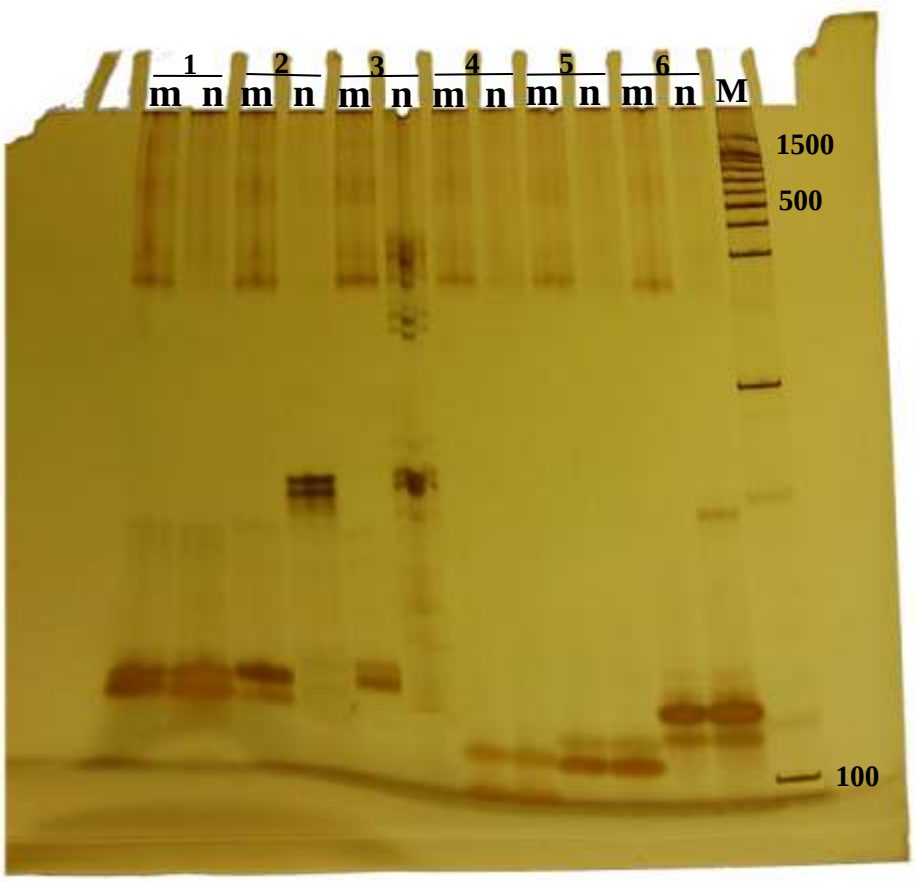


Figure 2.Image of Polyacrylamide gel representing SSR patterns in studiedgoat breeds obtained with microsatellite primers, (m) represents Merzi goat, (n) represents Native goats and (M) represents Ladder DNA.

(1): Primer OAR119. (3): Primer EP12 (5): Primer MC58
(2): Primer EP7. (4): Primer MC47 (6): Primer OAR266

The molecular weight of bands obtained from amplification of SSR (Simple Sequence Repeats) products of the investigated goat breeds ranged from 95 to178 bp (Table 3).Many distinct alleles observed that they can be indicative of possible breed marker, EP7 andEP12 were markers that can distinguish between the studied breeds Native and Merzi goats (Table 3).

Table (3). Loci and allele size in base pairs of studied goat breeds.

| Locus | Allele size(bp) | |
|---|---|---|
| | Native goat | Merzi goat |
| OAR119 | 117/128 | 117/128 |
| EP7 | 172/176 | 117/128 |
| EP12 | 175/178 | 123/128 |
| MC47 | 95/103 | 95/103 |
| MC58 | 101/105 | 101/105 |
| OAR266 | 109/115 | 109/115 |

The genetic relationship between the goat breeds was determined by using the genetic distances. The standard genetic distances were calculated for these populations with Nei's (1972) standard genetic distance (Ds).Close genetic distance was observed between Native And Merzi goats (D=0.1667)). The close similarity of Native and Merzi goats can be the result of gene flow from a common source. The result of phylogenetic tree (dendrogram)


Figure 3.Dendrogram showing genetic distances between Native and Merzi goats.

Was consistent with the background of the origin and geographical location of these breeds (Figure 3). The close kinship between Native and Merzi goats suggest some past crossing between these two geographical close populations.

In studies conducted in Iran on genetic distance between three goat breeds, it was found that a panel of 13microsatellites was sufficient to detect the differences between goat breeds (Mahmoudi *et al*., 2010). The reliability of microsatellite can thus contribute to the accurate identification of breeds. Also, in East Africa, studies were performed to evaluate the genetic relationship between indigenous goat breeds on the basis of microsatellite DNA markers. The results revealed that genetic distances between populations reflect their geographical proximity rather than morphological classification (Muema *et a*l., 2009).

Genetic markers are not only useful for measuring genetic distance between populations but they may also be used in measuring the similarity of individual genotypes with populations. Genetic similarity is a useful method of classifying individuals and populations based on marker genotype information. Further investigation is needed to study the exact properties of this new approach in populations of common origin and inbreed lines over generations.

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**توصيف نوعين من سلالات الماعز في محافظة دهوك باستخدام تقنية التتابعات البسيطة المتكررة**

**الخلاصة:**

في هذه الدراسة، استخدمت إحدى الطرق الجزيئية لتحديد بصمة ألدنا(DNA)لنوعين من الماعز في محافظة دهوك. تم جمع العينات من نوعين من الماعز ( الماعز المحلي ومرزي)، حيث أخذت العينات من مناطق مختلفة في محافظة دهوك. تم استخلاص ألدنا المجيني من الدم ونفذ تفاعل البلمرة المتسلسل(PCR) باستعمال بادئات خاصة. تم ترحيل عينات ألدنا على هلام الاكاروز وبعد ذلك تم ترحيلها على هلام البولي اكريلامايد 6% وصبغ الهلام بنترات الفضة. أظهرت النتائج بان هناك طرز مختلفة من حزم ألدنا والتي تعبر عن الصفات الوراثية الخاص بكل سلالة، اظهر التحليل الوراثي بان البعد الوراثي بين السلالتين كان بقيمة 0.1667 ، ان النتائج التي توصلنا اليها بمكن الاعتماد عليها في وضع برامج وخطط تهدف إلى تطوير واقع الثروة الحيوانية في هذا البلد .

**كار ئينانا فين گەر برينتا DNA ژ بو دیاركرنا دوجوری بزنا ل باریزگەها دهوکی**

**پوختە:**

**ئە ڤ ڤەكولینە هاتە بجیهینان ب گومكرنا چەند سامپلێن خوینی ژدوو جورێن جودا ژ بزنێن عیراقێ (بزنین خومالی و مەرزی ) سامپل هاتن وەرگرتن ژدهوكی. كەرەستێ الدنا مجینی هاتە وەرگرتن . تەفاعلین PCR هاتن ئەنجامدان و بشتی هینگێ سامپل هاتن بكارئینان ل سەر 6٪ پولی ئهاكریلاماید جیل. دوربونا جینیتكی ناف بەینا هەر دوو جورێن بزنا دیاربو بە زمارا 0.1667 ، ئەنجامێن ڤەكولینێ دبیت ببنە بناغەك وبنەرەتەك ژبو دانانا بروگرام وپیلاناژ بو بیشڤەبرن و چاككرنا سامانەگیانەوری ل وەلاتی.**