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Genotype and Allelic Frequencies of a Newly Identified Mutation Causing Blindness in Jordanian Awassi Sheep Flocks

K. I. Z. Jawasreh*, H. Ababneh[1], F. T. Awawdeh[1], M. A. Al-Massad[2] and A. M. Al-Majali[3]

Department of Animal Production, Faculty of Agriculture-Jordan University of Science and Technology (JUST),
P. O. Box (3030) IRBID 22110, Jordan

ABSTRACT : A total of 423 blood samples were collected (during 2009 and 2010) from all the ram holdings at three major Jordanian governmental Awassi breeding stations (Al-Khanasry, Al- Mushairfa and Al-Fjaje) and two private flocks. All blood samples were screened for the presence of mutations at the CNGA3 gene (responsible for day blindness in Awassi sheep) using RFLP-PCR. The day blindness mutation was detected in all studied flocks. The overall allele and genotype frequencies of all studied flocks of the day blindness mutation were 0.088 and 17.49%, respectively. The genotype and allele frequencies were higher in station flocks than the farmer flocks (0.121, 24.15 and 0.012, 2.32, respectively). Al-Mushairfa and Al-Khanasry stations have the highest genotype and allele frequencies for the day blindness mutation that were 27.77, 30.00% and 0.14, 0.171, respectively. The investigated farmer flocks have low percentages (0.03, 5.88% at Al-Shoubak and 0.005 and 1.05%, at Al-Karak, respectively for genotype and allele frequencies) compared with the breeding stations. Ram culling strategy was applied throughout the genotyping period in order to gradually eradicate this newly identified day blindness mutation from Jordanian Breeding station, since they annually distribute a high percentage of improved rams to farmer's flocks. (**Key Words :** Awassi Sheep, Day Blindness, CNGA3 Gene, PCR- RFLP)

INTRODUCTION

Sheep breeding is an ancient occupation in Palestine and its neighboring countries where it has been practiced for a thousand of years (Epstein, 1985). Improved Awassi was developed through breed selection and now the improved Awassi can be characterized by its high milk production (an average of 506 L/lactation) (Gootwine and Pollott, 2000). The improved Awassi have been distributed from Israel (the country of origin) to many countries around the world (Rummel et al., 2005). Sheep population in Jordan was estimated to be 2.5 million heads; the majority of this population is Awassi sheep that is the most dominant breed of sheep found in Jordan. In addition to the Improved Awassi that was imported from Israel starting from 1999 (MOA, 2008), some exotic breeds were imported from outside Jordan, such as Suffolk, Romanov Charollais, that reared for research purposes at Jordan University of science and Technology (JUST).

The improved Awassi in Israel can be found only in Ein Harod (the origin flock) which is the main source of this line of Awassi, and as Gootwine et al. (2008) reported that for several decades the Ein Harod dairy flock has been a closed flock. Only five Booroola Merino rams as foreign genetic material was introduced once in 1986, except this exotic rams, the replacement were selected from the same flock for several decades, which lead to increase the inbreeding that play a vital role in manifestation of undesired recessive traits, Shamir et al. (2010) showed the segregation of a novel recessive mutation that cause day blindness in Improved Awassi and recently Reicher et al. (2010) found the mutation that cause the day blindness in Awassi sheep, which is located in the CNGA3 gene that codes for the cone photoreceptor cyclic nucleotide-gated channel subunit α. Komáromy (2010) investigate the necessity of the availability of large animal models for both CNGA3- and CNGB3-achromatopsia for translational

* Corresponding Author : Khaleel Jawasreh. Tel: +962-7201000, Fax: +962-27095123, E-mail: kijawasreh@just.edu.jo
[1] National Center for Agricultural Research and Extension (NCARE), Jordan.
[2] Department of Animal Production Faculty of Agriculture, Jerash University, Jordan.
[3] Department of Veterinary Clinical Sciences, Faculty of Veterinary Medicine, Jordan University of Science and Technology, Jordan.
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purposes, while Kohl et al. (2005) shows that the majority of achromatopsia disease patients mainly affected by the mutations in these two genes (that are responsible for the disease). The mutation in CNGA3 gene affects negatively sheep breeding programs (increasing culling percentage). The distribution of improved Awassi through breeding station and stock owners flocks as well (MOA, 2008) may have a high contribution in distributing of some rams from the stations to farmers fields that may carry the undesired mutation of day blindness, and the eradication of this mutation from the breeding stations is the main step that can be done in order to avoid spreading of this mutation through breeding flocks and farmers flocks as well.

The main objective of this paper is to estimate the genotype and allele frequencies of the day blindness mutation in the improved and cross bred (Local×Improved) Awassi ram in the Hashemite kingdom of Jordan.

MATERIALS AND METHODS

Animals and sample collection

This study was conducted at Jordanian breeding stations (Al-Mushairfa, Al-Fjaje and Al-Khanasry stations) located in the northern and southern part of Jordan). All studied flocks consist of improved Awassi in addition to the local Awassi breed. A total of 423 blood samples were collected (during 2009 and 2010) from all the rams presented at the three major Jordanian governmental Awassi breeding stations. Two hundred ninety four samples were collected from the three governmental stations and 129 samples were collected from private flocks. The pedigree of all rams reared in the stations is available (unrelated animals were genotyped) but those who belong to farmers were with unknown ancestors. The blood samples were collected from Jugular vein of rams or ram lambs in 5 ml EDITA tubes and stored in the refrigerator (-20°C) until Lab. work.

DNA extraction

The frozen blood was thawed under room temperature and EZ -10 spin column kit (Bio basic) was used for DNA extraction as following; in order to deposit the white blood cells, 0.5 ml of whole blood was centrifuged at 3,000 rpm for 3 min at 4°C, then 0.8 of TBP buffer was added to break red blood cells. Then TBM buffer was added for breaking the white blood cells. After that proteinase K was added for breaking the protein and cell wall. Incubation step must follow at 55°C for 30 min. Absolute ethanol was also used for binding the DNA with cilica membrane. The following step was conducted by applying the mixture to EZ-10 columns. After that a centrifugation steps must be done and finally elution buffer was used for the purpose of depositing the DNA from Cilica membrane. Agarose gel 1.5% monitoring method was used for determination of quality of DNA. The Quantity of DNA was measured using S2100 UV/VIS DIODE-Array-spectrophotometer machine Version 1.7.

Genotyping process

The genotyping process was conducted at the National Center for Agricultural Research and Extension (NCARE)-Livestock Biotechnology Lab, Jordan. At the beginning of the genotyping process some samples were genotyped at the Institute of Animal Science ARO, the Volcani Center (Israel) by sending 175 blood samples to their then the procedure was adopted and optimized and starts running at the NCARE- Livestock biotechnology Lab. PCR-RFLP method was carried out for genotyping of the day-blindness mutation in CNGA3 gene using the primers designed by Reicher et al. (2010) that are: sense primer 5-TTCGATGAAGCTCCAGTCC-3′; antisense primer 5′-CTCACCTGGTCCGGGCTC-3′, (GenBank accession no. FN377574). The reaction mixture of PCR was performed by using 12.5 μl of master mix (Promiga Madison WI USA) with 1 μl of each of sense and antisense primer and 1 μl of genomic DNA and the volume was completed to 25 μl (tube volume) by sterilized water. The mixture was placed in thermal cycler (XP Cycler Bioer) the conditions of the thermal cycler were: the initial denaturation temperature step at 95°C for 5 min followed by 35 cycles of 95°C for 30 s, 65°C for 1 min, 72°C for 1 min and a final extension at 72°C for 5 min. PCR products were digested with *AvaII* restriction enzyme and size-separated and visualized by 12% poly acryl amide gel electrophoresis at the Volcai Center 3.5% Metaphor agarose gel electrophoresis was used for size separating and visualization. Genotype and allelic frequencies were calculated using Falconer and Mackay (1996) formula.

RESULTS AND DISCUSSION

The amplification of CNGA3 gene in PCR produced 107 bp band, while the digestion of this product by *Ava*II restriction enzyme harvest genotypes: homozygous normal-vision (RR) lamb; A major band of 41+45 bp and a minor band of 21 bp were detected. Heterozygous carrier (RS) lamb; Major bands of 66 bp and 41+45 bp were detected, along with a faint minor band of 21 bp (Figure 1). The homozygous day-blind lamb; Two major bands that supposed to be detected that reported by Reicher et al. (2010) to have one of 66 bp and the other of 41+45 bp. In our case no blind animals were genotyped because all blind born lambs culled early from the stations or flocks.

Out of 423 genotyped rams, 74 rams were found to be carriers for the mutation. Mutated allele and genotype

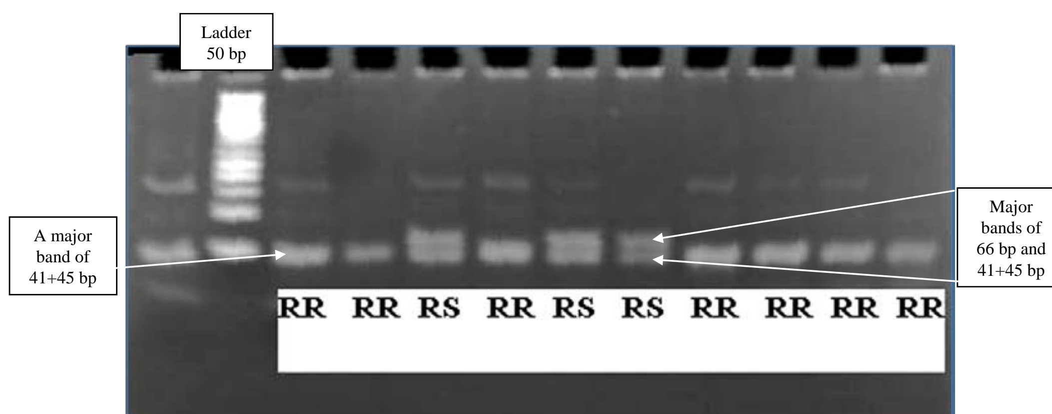

Figure 1. Genotyping for day blindness using 12% Acryl amide gel electrophoresis. RR: non affected; RS: Heterozygous for the day blindness mutation L. Ladder 50 bp.

frequencies (RS) were 0.088 and 17.49%, respectively (Table 1).

In stations this mutation has been identified to be with high RS (0.121%) and genotype frequency (24.15%) compared with lower frequencies in farmers' fields (Table 1). This mutation was higher at Al-Khanasry and Al-Mushairfa stations compared to Al-Fjaje station (Table 1). The distribution of improved rams to the farmers (the strategy of Ministry of Agriculture) for genetic improvement of the local Awassi breed may have a substantial contribution in the distribution of this mutation in private flocks, but still in low level compared with the situation in the breeding stations (Table 1).

The frequency of day blindness mutation in Jordan was higher than that reported by Shamir et al. (2010) (1.7%) in Israeli improved Awassi flocks. The mutated allele (RS) frequency in Jordan was lower than RS frequency in Israeli improved ram (0.35) and ewes (0.25) that genotyped at the Volcai Center (unpublished data). The only work that conducted in day blindness of improved Awassi that published by the Israeli group (Shamir et al., 2010; Reicher et al., 2010).

The distribution of the improved Awassi rams through breeding stations and farmers flocks has been detected as main risk factors for the distribution of the mutation within Awassi flocks.

Eradication program must be continued in order to decrease the frequency of the unfavorable allele in breeding station as the first step that can be lead to avoiding having such affected rams in farmer's fields.

CONCLUSION

Allele and genotype frequencies were high in Jordanian breeding stations and culling program must be adopted and maintained for the purpose of diminishing the rate of dispersion of this unfavorable mutation in governmental breeding stations and private sheep flocks.

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Table 1. Overall frequency of carriers of the day-blindness mutation in Jordanian Awassi Rams flocks

Flock's	Farm	No. of rams genotyped	No. of carriers	Mutation allele frequency	Genotype frequency (%) mutation carriers
Overall	All locations	423	74	0.088	17.49
Stations	All stations	294	71	0.121	24.15
	Al-Fjaje	66	5	0.037	7.58
	Al-Mushairfa	108	30	0.140	27.77
	Al-Khanasry	120	36	0.171	30.00
Farmers' fields	All locations	129	3	0.012	2.32
	Al-Karak	95	1	0.005	1.05
	Al-Shoubak	34	2	0.030	5.88

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