IDENTIFICATION OF FECUNDITY GENE IN EGYPTIAN GOATS USING GENETIC MARKERS

I. BIOCHEMICAL POLYMORPHIC MARKERS

M. R. ANOUS[1], M. A. RASHED[2], H. R. MOTAOA[3], M. H. SADEK[1], Y. M. SAAD[4], M. A. OSMAN[3] AND E.M. SHATH[3]

1. *Department of Animal Production, Faculty of Agriculture, Ain Shams Univ., Cairo, Egypt*
2. *Department of Genetics, Faculty of Agriculture, Ain Shams University, Cairo, Egypt*
3. *Animal Production Research Institute, Sheep and Goat Research Division, Dokki, Giza, Egypt*
4. *National Inst. of Oceanography and Fisheris, Genetic Laboratory, El-Kanatair, Kaluobeia, Egypt*

Goats distribute widely all over the world. They thrive in a wide variety of environmental conditions. They are also considered as one of the important economic sources of meat in developing countries. Improving the reproductive efficiency of goat herds in these countries, can increase the efficiency of kid production and, consequently, goat meat.

Therefore, introducing the gene responsible for the high reproductive rate or prolificacy (i.e. number of individuals born per parturition per female = litter size), known as Booroola gene (or FecB gene) in sheep (Piper and Bindon, 1996), from prolific goat breeds into non-prolific goat herds may increase reproductive rate without substantial changes in other economically important traits.

On the other hand, the polymorphism of blood genetic markers gives some useful information in studies of animal breeding such as the relationships among breeds and their evolution. It can also be used for indirect selection if there were some relationships between these markers and some economically important quantitative traits. In this case, many researchers employed classical biochemical polymorphic markers such as polymorphic proteins (Wang *et al*., 1990; Machado *et al*., 2000; Deza *et al*., 2000; Han and Yuzhu, 2002) and isozymes (Di Stasio *et al*., 1995; Cengiz and Asal, 2000; Menrad *et al*., 2002; Nyamsamba *et al*., 2003) to characterize and estimate genetic distances between breeds of goats and also in the study of genetic diversity within goat breeds. These markers were also used to reconstruct the phylogenetic relations among goat populations. These methods are isoelectric focusing (Machado *et al*., 2000) and SDS-PAGE (Laemmli, 1970).

In Egypt, there are many native goat breeds with different productive and reproductive performances. The most important of them are the Baladi, mainly reared for meat production, and the Nubi (or Zaraibi), and mainly reared for milk production. They both are characterized by a good prolificacy (i.e. litter size), but

little is known about their genetic relationship at both biochemical and molecular genetic levels. The research to locate the gene responsible for the prolificacy in these two breeds may help its use commercially to improve other local non-prolific breeds, especially the Barki breed, mainly reared for meat production. Results of the research work reported by Marai *et al*. (2001) revealed the existence of association between reproductive traits of Nubian doe genotypes and marker gene alleles.

Therefore, the present study was conducted for the assessment of the genetic structure within each of these three Egyptian goat populations using some biochemical genetic markers (i.e. serum proteins and isozymes) in order to find genetic markers which can differentiate between them.

MATERIALS AND METHODS

I. Animal pedigree records and data collection

The base goat population was assembled in 2006 from three different experimental stations belonging to Animal Production Research Institute (Borg El-Arab, El-Serw and Sakha). A total of 91 females of different ages were collected from three Egyptian goat breeds: Barki (n = 22), Baladi (n = 21) and Zaraibi (n = 48), according to their litter size trait, using the pedigree records.

II. Animal groups construction

The individuals of the Zaraibi breed (n = 48), considered as prolific goats, were chosen from a total of 292 females raised in two farms (i.e. El-Serw and Sakha). They were assembled into two groups; prolific (n = 26) and non-prolific (n = 22) females, according to the number of kids born per parturition per female: three or more kids for the first group and one or two kids for the second group. Figure (1) shows the structure of the Zaraibi herd based on kid type of birth (i.e. number of kids born per parturition per female). However, individuals of the Baladi, considered as prolific goat, and Barki, considered as non-prolific goat, were assembled in only one group for each; prolific for the Baladi females (n = 21); chosen at random from a total of 30 females raised at Sakha station, and non-prolific for the Barki females (n = 22); chosen at random from a total of 45 females raised at Borg El-Arab station. All individuals were managed under the same conditions and they were healthy and in a good body condition.

III. Blood samples collection

Blood samples were collected from the selected animals by vacutainer glass tubes which contain disodium EDTA (EDTA-Na2) as anticoagulant reagent. They were taken from 63 females out of 91 of the selected animals: 22 does from the Barki breed, 21 does from the Baladi breed and 20 from the Zaraibi breed (13 and 7 prolific and non-prolific does, respectively, all taken from the herd of Sakha Station). We are not able at this time to get blood samples from the Zaraibi females of the Serw Station due to

the contamination of the farm with the foot and mouth disease.

Plasma serum was then obtained by centrifugation at 5000 rpm for 15 minutes at 4°C, and the plasma protein (supernatant) was transferred to clean plastic vials and stored at -20°C until the time of electrophoretic analyses.

IV. Genetic characterization using biochemical genetic markers (SDS-Protein PAGE and Isosymes)

1. SDS-Protein PAGE electrophoresis

Samples were applied to 15% polyacrylamid gel. Gel preparation, electrophoresis conditions, staining and destaining gels were done according to Laemmli (1970).

Protein fractionations were performed exclusively on vertical slab (19.8 cm x 26.8 cm x 0.2 cm) gel using the electrophoresis apparatus manufactured by Aplex.

2. Isozymes electrophoresis

The same goat blood serum samples were used for detecting isozyme polymorphisms within each of the three goat populations.

Two isozyme systems (i.e., Esterase and Malate dehydrogenase; Mdh) were applied. Isozymes electrophoresis were carried out according to Tanksley and Rick (1980) and Tanksley and Orton (1983).

V. Statistical analysis

1. Genetic identity and genetic distance

Both genetic identity and distance were estimated according to Bardacki and Skibinski (1994). The banding patterns of the individuals were compared within populations. Bands were scored as 1 if present and 0 if absent.

2. Dendrogram construction

For constructing a combined dendrogram for each goat breed, the data generated from protein banding patterns and isozymes variations were introduced to SPSS package program according to binary values (1, 0).

RESULTS AND DISCUSSION

A) Protein polymorphism

Protein electrophoretic banding patterns of the three collected goat populations are shown in Figs (2 and 3) for Zaraibi, Figs (4 and 5) for Baladi and Figs (6 and 7) for Barki. The dendrogram of the Zaraibi population is presented in Fig. (8). The similarity values were also calculated based on protein banding patterns for each population.

1. Zaraibi (Z) population

The protein electrophoretic banding patterns of Nubi (or Zaraibi) goat breed are shown in Fig. (2) for the prolific group (ZH) and Fig. (3) for the non-prolific one (ZL). A total of 25 bands were detected, according to their relative

fronts, based on band molecular weight (MW) along the gel. These MWs of protein bands ranged from 28 to 230 kDa. However, the range of MW of protein bands was from 28 to 179 kDa for ZH group and from 29 to 207 kDa for ZL group. The two Zaraibi goat groups were shared in two common bands at molecular weight of 92 kDa and 29 kDa, so these two bands could be used as specific protein markers to characterize this breed which is mainly reared for milk production.

According to protein polymorphism data, the ZH group has six common specific protein markers at molecular weights of 28, 31, 43, 53, 67 and 179 kDa, while the ZL group has seven common specific protein markers at molecular weights of 51, 69, 131, 138, 185, 203 and 207 kDa. So, the ZH specific protein markers could be considered as important markers to characterize the prolific females within this breed.

The average of similarity indices within ZH group was 0.86, while it was 0.76 within ZL one. On the other hand, the average of similarity indices within Zaraibi population from the pooled data (ZH and ZL together) was 0.55.

The dendrogram of this population (Fig. 8) showed that it was divided into two groups; the first represented the prolific females (n = 13) and the second represented the non-prolific ones (n = 7). The first group was also divided into two sub-groups, which reflected the existing variability in litter size trait within the females. This may explains the low value of similarity indices within the Zaraibi population, as a whole, and the high values of similarity indices obtained in both prolific and non-prolific sub-groups.

2. Baladi (B) population

The electrophoretic protein patterns of the individuals of this population are shown in Figs (4 and 5). A total of 32 bands were labeled according to their relative fronts. The MW of protein bands ranged from 28 to 211 kDa. The Baladi individuals had eight common protein markers at molecular weights of 28, 29, 31, 56, 119, 146, 198 and 206 kDa. The average of similarity indices within this population was 0.65.

3. Barki (R) population

The electrophoretic protein patterns of the individuals of this population are shown in Figs (6 and 7).

A total of 22 bands were labeled according to their relative fronts. The protein bands ranged from 24 to 208 kDa. The Barki individuals had only one common protein marker at molecular weight of 55 kDa. The average of similarity within this population was 0.69.

Thus, the protein electrophoresis (SDS-PAGE) in the present study indicated that each goat population had a unique banding pattern.

The average of similarity indices reflects the range of homogeneity within

each population. Relatively, the Barki population has the highest average value (0.69) followed by the Baladi population (0.65), while the Zaraibi population has the lowest value (0.55). This may reflect a higher degree of inbreeding in both the Barki and the Baladi populations compared to the Zaraibi one.

We can conclude that SDS-Protein PAGE is a sensitive method for studying the genetic structure of goat populations.

B) Isozymes polymorphism

Isozymes patterns for Esterase (Eat.) and Malate dehydrogenase (Mdh) were presented in Figs (9-18) and summarized in Tables (1-6). The similarity values were also calculated based on isozymes banding patterns for each of the three goat populations.

1. Zaraibi (Z) population

The electrophoretic patterns of Esterase and Malate dehydrogenase isozymes for the females of this population are shown in Figs (9 and 10) and summarized in Tables (1 and 2), respectively.

A total of 4 and 5 bands (Tables 1 and 2), according to their relative fronts, were detected in this goat breed for Est. at RF ranged from 0.12 to 0.87 and Mdh at RF ranged from 0.10 to 0.30, respectively. The average of similarity indices within this population for the two isozymes was 0.54.

2. Baladi (B) population

The electrophoretic patterns of Esterase and Malate dehydrogenase isozymes of the females of this population are shown in Figs (11-12 and 13-14), respectively and summarized in Tables (3 and 4).

A total of 3 and 3 bands (Tables 3 and 4), according to their relative fronts, were detected in this goat breed for Est. at RF ranged from 0.12 to 0.75 and Mdh at RF ranged from 0.10 to 0.32, respectively. The average of similarity indices within this population was 0.70.

3. Barki (R) population

The electrophoretic patterns of Esterase and Malate dehydrogenase for the females of this population are shown in Figs (15-16 and 17-18), respectively and summarized in Tables (5 and 6).

A total of 3 and 3 bands (Tables 5 and 6), according to their relative fronts, were detected in this goat breed for both Est. at RF ranged from 0.12 to 0.87 and Mdh at RF ranged from 0.10 to 0.22, respectively. The average of similarity indices within this population was 0.95.

According to the previous results, there are individual variations within each population especially within the Zaraibi one. These results confirmed the previous results obtained with the (SDS-Protein PAGE) method in that each goat population had a unique protein banding pattern.

The isozymes systems used in the present work are previously used in studying biochemical variations in Egyptian goat (Abu Shady, 2004) and in another goat breeds (Menrad *et al.*, 2002; Nyamsamba *et al.*, 2003). Abu Shady (2004) reported that Mdh was not able to distinguish among breeds, sexes or even between different locations, while the Esterases had a value to discriminate among goat populations. However, Nyamsamba *et al.* (2003) could not obtain any clear genetic differences among eight native goat populations by studying their isozymes variations. On the other hand, Deza *et al.* (2000) studied the isozymes variations (Mdh and Est.) among some native goats from different locations. They found individual differences among goat samples using these isozymes. In addition, Menrad *et al.* (2002) found the same conclusion after studying the genetic variability of goat plasma.

In general, the present work gave us an overview about the genetic variations within the three Egyptian goat populations based on biochemical markers (protein and isozymes markers). On the other hand, molecular techniques at the DNA level will be needed to detect more genetic markers for the prolificacy trait in Egyptian goats. These molecular markers and the protein markers will be used for markers assisted selection to increase returns per unit of production in goats.

SUMMARY

Blood samples were collected from 63 females, taken from three Egyptian goat breeds, by vacutainer glass tubes which contain disodium EDTA (EDTA-Na2) as anticoagulant reagent. Twenty-two does from the Barki breed, 21 from the Baladi breed and 13 and 7 prolific and non-prolific does, respectively from the Zaraibi breed, were chosen according to the litter size trait.

Blood serum was then obtained by centrifugation and treated by two bio-chemical fingerprints techniques; SDS-Protein PAGE and Isozymes, to characterize the three goat breeds and to find genetic markers which can differentiate between them.

The protein electrophoresis (SDS-Protein PAGE) in the present work indicated that each goat population had a unique protein banding pattern. The Barki population has the highest value of similarity (0.69) followed by the Baladi one (0.65), while the Zaraibi population showed the lowest value (0.55). However, the average of similarity within both the prolific (ZH) and non-prolific (ZL) Zaraibi groups was high (0.86 and 0.76, respectively).

The two Zaraibi goat groups shared two common bands at molecular weight of 92 kDa and 29 kDa, so these two bands could be used as specific protein markers to characterize this breed. For the Zaraibi breed, 6 protein markers were detected as specific ones for ZH females (at molecular weights of 28, 31, 43, 53, 67 and 179 kDa) and 7 protein markers were also detected as specific ones for ZL females (at molecular weights

of 51, 69, 131, 138, 185, 203 and 207 kDa). So, the ZH specific protein markers could be considered as important markers to characterize the prolific females within this breed. The Baladi individuals had eight common protein markers (at molecular weights of 28, 29, 31, 56, 119, 146, 198 and 206 kDa), while the Barki ones showed only one common protein marker at molecular weight of 55 kDa. So, these specific protein markers could be considered as important markers to characterize these two breeds.

A total of 4 and 5 bands, 3 and 3 bands and 3 and 3 bands were detected for Esterase (Est.) and Malate dehydrogenase (Mdh), respectively in Zaraibi, Baladi and Barki goats, respectively. The analysis of the two isozyme systems used in the present work showed that there are individual variations within each of the three goat populations; the greatest in the Zaraibi breed and the lowest in the Barki breed.

ACKNOWLEDGMENT

The authors are very grateful to the Program of the National Strategy for Biotechnology and Genetic Engineering of the Science and Technology Center, Academy of Scientific Research and Technology, Ministry of Scientific Research who funds and administrates this study through the research project No. 103 entitled "Detection of molecular markers linked to prolificacy in Egyptian goats".

REFERENCES

Abu Shady, A. M. (2004). Molecular genetic markers in the identification and improvement of goat breeds in Egypt. M.Sc. Thesis, Department of Genetics, Ain Shams University, Egypt.

Bardacki, F. and D. O. F. Skibinski (1994). Application of the RAPD technique in tilapia fish: species and subspecies identification. Heredity, 73: 123-177.

Cengiz, E. and S. Asal. (2000). Blood biochemical polymorphism in Angora goat breed. Egyptian J. Animal Production, 37: 31-36.

Deza, C., O. T. Perez, C. N. Gardenal, I. Varel, M. Villar, S. Rubiaies and C. Barioglio (2000). Protein Polymorphism in native goats from General Argentina. Small Ruminant Research, 35: 195-201.

Di Stosio, L., R. Rasivo, P. Piandra and P. Giaccone (1995). Polymorphism of erythrocyte malic enzyme in the goat AniiDli. Genetics, 26: 275-276.

Han, J. and L. Yuzhu (2002). Study on the polymorphism of transferrin of native Zhongwei goats, crossbreeds of Angora x Zhongwei (F_1) and Angora x F_1. Proceedings of 7th world congress on genetics applied to livestock production, Aug. 19-23, Montpellier, France.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680-685.

Machado, T. M. M., M. L. S. P. Igarashi, E. P. B. Contel and J. A.Ferro (2000). Genelic diversity within the goat populations of Brazil. Proceeding of 7th International Conference on Goats, France, 15-21 May, p. 958-960.

Marai I. F. M., E. I. Abou-Fandoud, A. H. Daader and A. A. Abu-Ela (2001). Association between marker gene alleles and doe traits in Nubian (Zaraibi) goats in Egypt. Annals of Arid Zone, 40: 193-197.

Menrad, M., C. H. Stier and C. F. Gall (2002). A study on the Changthangi Pashmina and the Baker wali goat breeds in Kashmir I. Analysis of blood protein Polymorphisms and genetic variability within and between the Populations. Small Ruminant Research, 43: 3-14.

Nyamsamba, D., K. Noniura, M. Nozawa, K. Yokohama, Yo. Zagdsuren and T. Ainano (2003). Genetic relationship among Mongolian native goat populations estimated by blood protein polymorphism. Small Ruminant Research, 47: 171-181.

Piper, L. R. and B. M. Bindon (1996). Prolific Sheep. The Booroola Merino. In: Fahmy, M.H. (Ed.), Agric. and Agri-Food Canada, Lennoxville, Quebec, Canada, p. 152-160.

Tanksley, D. and C. Rick (1980). Genetics of esterases in species of Lycoporicon. Theor. Appl. Genet., 56: 209-219.

Tanksley, D. and T. Orton (1983). Isozymes in plant genetic and breeding. Part (B). Elsevier Sci. Publishers B. V. Amsterdam.

Wang, S., W. C. Fiiotc and T. I. Hunch (1990). Transferen and hemoglobin polymorphism in domesticated goats in the USA. Animal Genetics, 21: 91-94.

Table (1): Esterase isozymes polymorphism for Zaraibi goat population.

BN	RF	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	0.12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	0.32	1	1	0	0	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	0
3	0.62	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	1	0	0
4	0.87	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

0 = absent, 1 = present, BN= Band number, RF= Relative front.

Table (2): Mdh isozymes polymorphism for Zaraibi goat population.

BN	RF	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	0.10	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0
2	0.14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
3	0.18	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1
4	0.20	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1	0	0	0	0
5	0.30	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

0 = absent, 1 = present, BN= Band number, RF= Relative front.

Table (3): Esterase isozymes polymorphism for Baladi goat population.

BN	RF	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	0.12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	0.37	0	1	0	0	0	0	0	1	0	0	0	1	1	1	0	0	0	0	1	1	1
3	0.75	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

0 = absent, 1 = present, BN= Band number, RF= Relative front.

Table (4): Mdh isozymes polymorphism for Baladi goat population.

BN	RF	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	0.10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	0.22	0	0	0	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
3	0.32	1	1	1	1	0	0	0	1	1	1	1	1	1	1	1	0	0	0	1	1	1

0 = absent, 1 = present, BN= Band number, RF= Relative front.

Table (5): Esterase isozymes polymorphism for Barki goat population.

BN	RF	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	0.12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	0.37	1	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
3	0.87	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

0 = absent, 1 = present, BN= Band number, RF= Relative front.

Table (6): Mdh isozymes polymorphism for Barki goat population.

BN	RF	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	0.10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	0.15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
3	0.22	1	1	1	1	1	0	1	0	0	0	1	1	0	1	1	1	1	1	1	1	1	0

0 = absent, 1 = present, BN= Band number, RF= Relative front.

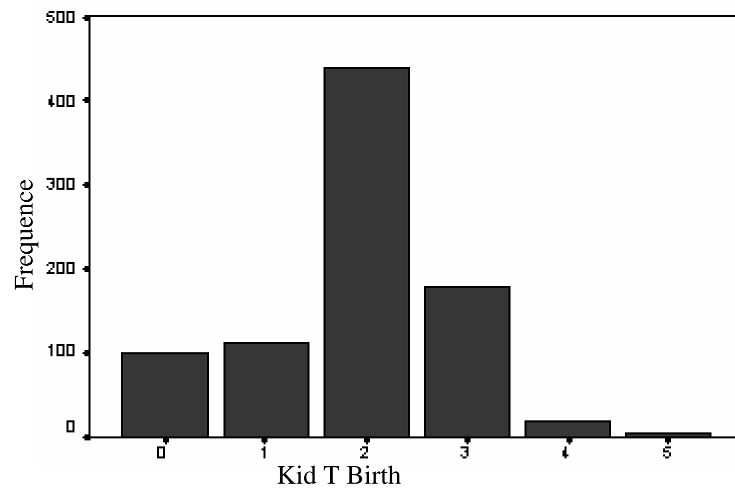


Fig. (1): Kid type of birth distribution for the available Zaraibi females.

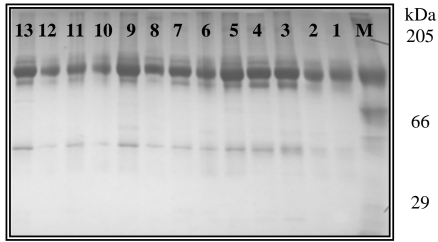


Fig. (2): SDS-PAGE profile of Zaraibi goat (prolific females) serum proteins and M= protein markers.

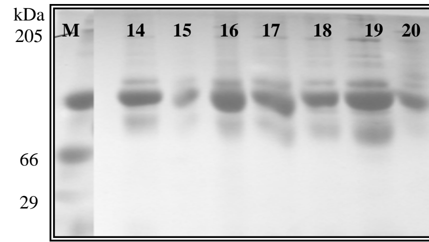


Fig. (3): SDS-PAGE profile of Zaraibi goat (non-prolific females) serum proteins and M= protein markers.

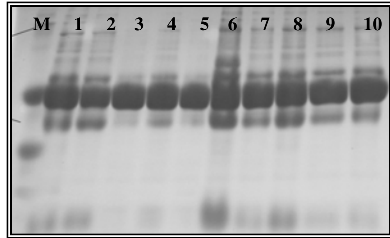


Fig. (4): SDS-PAGE profile of Baladi (B) goat serum proteins (samples from 1-10) and M= protein markers.

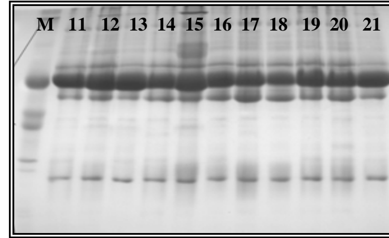


Fig. (5): SDS-PAGE profile of Baladi (B) goat (samples from 11-21) serum proteins and M= protein markers.

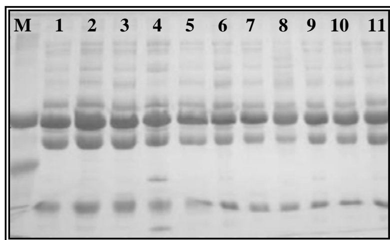


Fig. (6): SDS-PAGE profile of Barki (R) serum proteins (samples from 1-11) and M= protein markers.

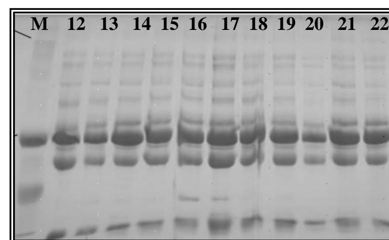
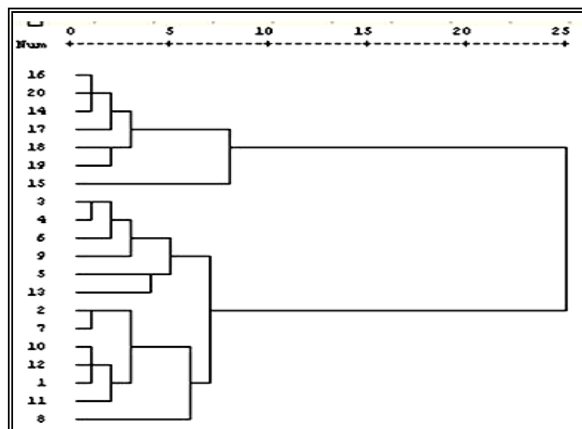


Fig. (7): SDS-PAGE profile of Barki (R) serum proteins (samples from 12-22) and M= protein markers.

Fig. (8): Dendrogram of genetic relationship among the 20 Zaraibi individuals.



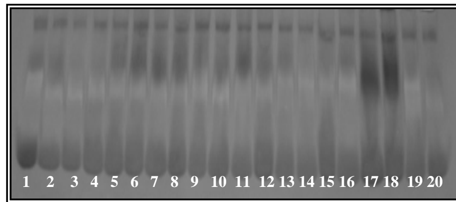


Fig. (9): Esterase electrophoretic patterns of Zaraibi goat (1-13 are ZH 14-20 are ZL).

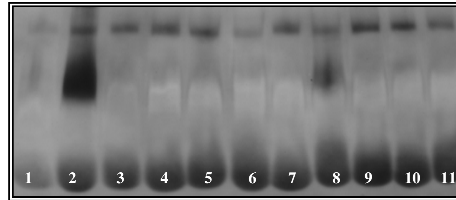


Fig. (11): Esterase electrophoretic patterns of Baladi goat (samples 1-11).

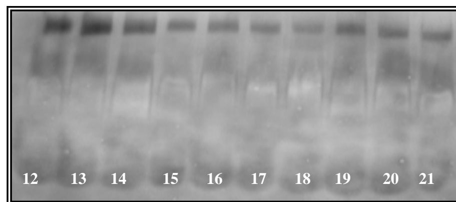


Fig. (12): Esterase electrophoretic patterns of Baladi goat (samples 12-21).

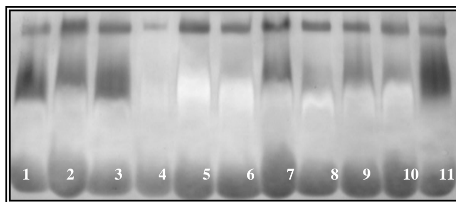


Fig. (15): Esterase electrophoretic patterns of Barki goat (samples 1-11).

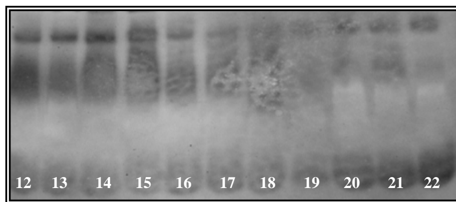


Fig. (16): Esterase electrophoretic patterns of Barki goat (samples 12-22).

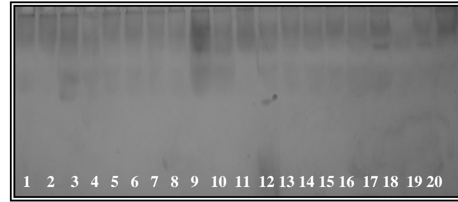


Fig. (10): Mdh profile of Zaraibi goat (1-13 are prolific and 14-20 are non-profilic).

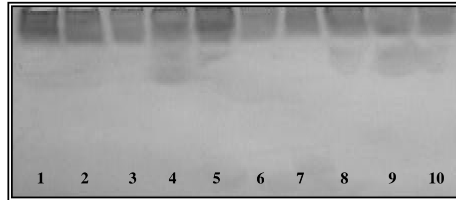


Fig. (13): Mdh profile of Baladi goat (samples 1-10).

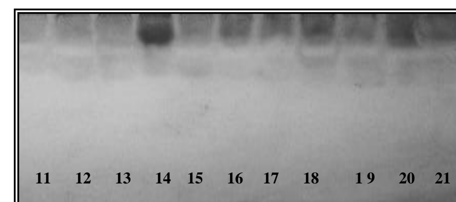


Fig. (14): Mdh profile of Baladi goat (samples 11-21).

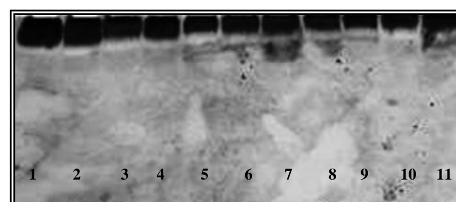


Fig. (17): Mdh profile of Barki goat (samples 1-11).

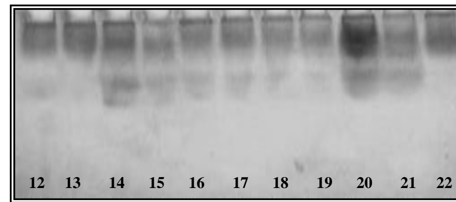


Fig. (18): Mdh profile of Barki goat (samples 12-22).