PARENTAGE TESTING OF ARABIAN HORSE IN EGYPT USING MICROSATELLITE DNA TYPING

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A B S T R A C T

This study was carried to establish a parentage testing system for the Arabian horses in Egypt. Thirty-eight Arabian horse samples including seventeen foals, five sires and sixteen dams from El Zahraa stud were genotyped by using seventeen microsatellite markers recommended by the International Society for Animal Genetics (ISAG). The number of alleles varied from 4 (ASB23, HMS2) to 13 (VHL20). The observed heterozygosity and expected heterozygosity ranged from 0.27 (HMS3) to 0.816 (HTG10) and from 0.474 (ASB23) to 0.841 (ASB17) respectively. PIC value ranged from 0.423 (ASB23) to 0.812 (ASB17) with average 0.650. There was 100 % matching between pedigree and DNA typing parentage for the 17 examined foals. These results suggested that the DNA typing method had high potential for parentage verification and individual identification of the Arabian horses in Egypt.

Key Words: Parentage test, Arabian horses, Egypt and microsatellite.

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1. INTRODUCTION:

According to the Arabian Horse Association (AHA), the five primary strains were known as the Keheilan, Seglawi, Abeyan, Hamdani and Hadban. There were also lesser strains, sub-strains, and regional variations in strain names. Purity of bloodline was very important to the Bedouin, Carl Raswan, a promoter and writer about Arabian horses from the middle of the 20th century, held the belief that there were only three strains, Kehilan, Seglawi and Muniqi. Raswan felt that these strains represented body "types" of the breed, with the Kehilan being "masculine", the Seglawi being "feminine" and the Muniqi being "speedy"(Siegal, 1996). The origin of the Arabian horse in the desert remains a matter of study and controversy. Contemporary Arabian horses descend from stock bred by nomadic Bedouin tribes in Arabia Deserta, an area covering most of what is now Syria, Jordan, Iraq, Saudi Arabia, the United Arab Emirates, Qatar, Bahrain, Kuwait, Yemen, and Oman, which were called the "countries of origin."(Arabian horse History, 2014). The need for paternity testing increases specially in natural breeding that had multiple estrus or when using artificial insemination (AI) and the original sir is not well known. Breed registry authorities have adopted parentage testing programs worldwide to assure horse pedigree integrity, which is essential for breeding management plans. (Marshall et al., 1998) The most efficient genetic markers for such use are co-dominant and inherited by Mendelian principles. Traditionally, in most laboratories, a battery of 14 to 17 systems (7 blood groups and 10 protein polymorphisms) has been used(Bowling, 1997).But now microsatellite markers are widely accepted as a marker of choice as they are highly polymorphic single locus

DNA sequenced throughout the genome and are readily adaptable to Polymerase Chain Reaction (Tozaki, 2001). Microsatellite is the common term, which is used to describe repetitive tandem, repeats of short simple sequence motifs, and not more than six bases long. Microsatellites show high level of polymorphism (Litt and Lutty, 1989) and are uninterrupted array (Weber and May, 1989). Microsatellite markers are useful to study genetic variation, parentage assessment, and study of gene flow and to measure genetic distance between two or more breeds or related species (Bruford and Wayne, 1993). Therefore, this study is an attempt for parentage verification and individual identification of Arabian horse in Egypt using seventeen sets of equine microsatellite markers.

2. MATERIALS AND METHODS:

2.1. Animals and DNA extraction:

Genomic DNA was extracted from hair roots, which were collected from 38 horses including 17 foals using EZ10 Spin Genomic DNA Minipreps, Animal (cat. No: BS427 - Bio Basic Inc - Canada) according to the manufacturer's protocols.

2.2. Microsatellite markers genotyping.

Seventeen microsatellites were selected for this study that had been reported by the horse applied genetics committee of ISAG for individual identification and parentage verification of Arabian horses. Microsatellite markers (table 1) were combined in multiplex PCR reaction using fluorescently labeled primers and amplified in a total volume of 15 µl of the following mixture: 1ul(5 ng) of genomic DNA, 4 µl primer mix, 4 µl of dNTPs, 2.5 µl of stockmark PCR buffer, and 0.5 µl of ampliTaq Gold DNA polymerase (Applied Biosystems, USA). PCR amplification was as follows: first step was performed by initial denaturation for10 min at 95ºC, followed by 30 cycles at 95ºC for 30 sec, 60oC for 30 sec, and 72oC for 1 min then extension step of 72ºC. Fragments were further sequenced using Genetic analyzer 3500 (Applied Biosystem-USA) with the aid of Liz standard. The data obtained is further analyzed using Gene Mapper V 4.1 software (Applied Biosystem)

2.3. Data analysis

Number of alleles (*NA*), observed heterozygosity (H_O), expected heterozygosity (H_E) and Polymorphic information content (PIC) were calculated by using CERVUS version 3 software (Kalinowski *et al.* 2007). Hardy Weinberg Equilibrium (*HWE*) was estimated by GENEPOP version 3.4 program (Raymond and Rousset 1995). Allele's frequencies for each locus were calculated by using GENALEX version 6 software (Peakall and Smouse 2006).

The Proposed nomenclature for the 17 equine short tandem repeat loci investigated is based on the number of repeat units and is adopted from the recommendation of International Society of Forensic Genetics (ISFG) for the nomenclature of human STRs (Bozzini et al., 1996).

3. RESULT:

Microsatellites were highly polymorphic in Arabian horses (Table 2). The average number of alleles is (7.563). The number of alleles varied from 4(ASB23, HMS2) to 13 (VHL20). The observed heterozygosity and expected heterozygosity ranged from 0.27 (HMS3) to 0.816 (HTG10) and from 0.474 (ASB23) to 0.841 (ASB17) respectively. PIC value ranged from 0.423 (ASB23) to 0.812 (ASB17) with average 0.650. Allele's frequency of 17 microsatellite markers were shown in figures (1 to 17). The highest number of alleles was 13 for VHL20 locus as shown in figure (2). The lowest number of alleles was 4 for ASB23 and HMS2 loci as shown in figures (9 and 10) respectively. The parentage verification of the 17 foals was qualified by the compatibility of 17 microsatellite markers according to Mendelian fashion as shown in table 3.

Table (1): Characteristics of 17 equine microsatellites DNA loci :

Locus	Dye	Color	Expected Size Range (bp)[a]
VHL20	6-FAM™	Blue	83–102
HTG4	6-FAM	Blue	116–137
AHT4	6-FAM	Blue	140–166
HMS7	6-FAM	Blue	167–187
HTG6	VIC®	Green	74–103
AHT5	VIC	Green	126–147
HMS6	VIC	Green	154–170
ASB23	VIC	Green	176–212
ASB2	VIC	Green	237–268
HTG10	NED™	Yellow	83–110
HTG7	NED	Yellow	114–128
HMS3	NED	Yellow	146–170
HMS2	NED	Yellow	215–236
ASB17	PET®	Red	104–116
LEX3	PET	Red	137–160
HMS1	PET	Red	166–178
CA425	PET	Red	224–247

Table (2) Number of alleles (N_A), observed (H_O) and expected (H_E) heterozygosities, polymorphic information content (PIC) and Hardy Weinberg Equilibrium (HWE) of 16 microsatellites loci for Egyptian Arabian horse.

Locus	Na	N	Ho	He	PIC	HWE
VHL20	13	38	0.737	0.838	0.808	*
HTG4	6	38	0.763	0.699	0.646	**
AHT4	9	38	0.789	0.801	0.761	NS
HMS7	7	38	0.789	0.757	0.711	NS
HGT6	9	38	0.632	0.68	0.629	NS
AHT5	7	38	0.763	0.797	0.758	NS
HMS6	5	38	0.789	0.687	0.613	NS
ASB23	4	38	0.447	0.474	0.423	NS
ASB2	6	34	0.676	0.706	0.66	NS
HTG10	9	38	0.816	0.793	0.754	**
HTG7	6	38	0.711	0.628	0.58	NS
HMS3	7	37	0.27	0.613	0.564	***
HMS2	4	33	0.515	0.651	0.569	NS
ASB17	12	38	0.526	0.841	0.812	***
Lex 3	9	38	0.579	0.752	0.706	*
HMS1	9	36	0.306	0.5	0.47	***
CA425	8	37	0.568	0.68	0.635	NS
	7.563	37.188	0.631	0.697	0.650	

Table (3) Example of case of parentage test combinability by 17 microsatellite loci:

		AHT4	**AHT5**	**ASB2**	**ASB17**	**ASB23**	**CA425**	**HMS1**	**HMS2**	**HMS3**	**HMS6**	**HMS7**	**HTG4**	**HTG6**	**HTG7**	**HTG10**	**LEX3**	**VHL20**
Case1	sire	L/P	M/N	Q/Q	M/R	I/I	L/M	I/M	L/L	P/P	L/M	N/N	K/M	M/M	K/P	K/R	-	R/R
	Foal	P/P	M/N	Q/Q	M/R	I/J	L/L	I/J	L/L	P/P	L/L	N/N	M/M	M/M	K/O	K/R	H/-	L/R
	Dam	K/P	M/O	Q/Q	R/R	J/J	L/N	J/M	L/L	P/P	L/M	L/N	K/M	M/M	K/O	K/O	H/-	L/R

Alleles frequency of 17 microsatellite markers were shown in figures (1 to 17):

Alleles frequency of 17 microsatellite markers were shown in figures (1 to 17):

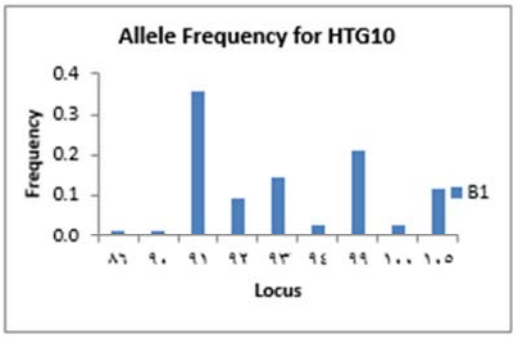


Figure 1: Allele frequency of HTG10

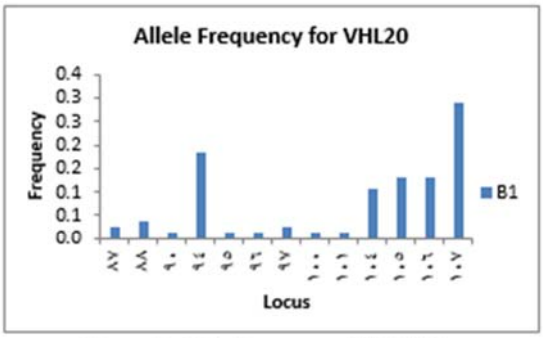


Figure 2: Allele frequency of VHL20

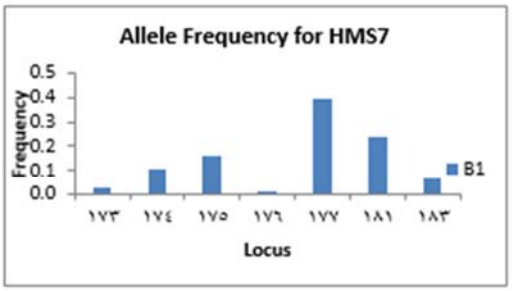


Figure3 : Allele frequency of HMS7

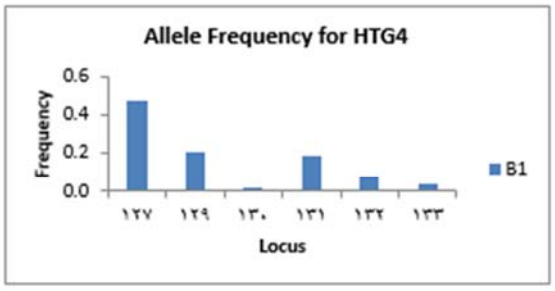


Figure4 :Allele Frequency of HTG4

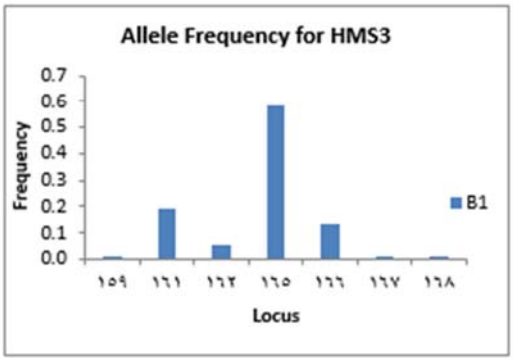


Figure5 : Allele frequency of HMS3

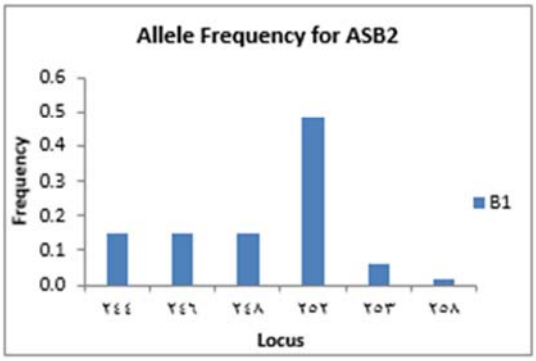
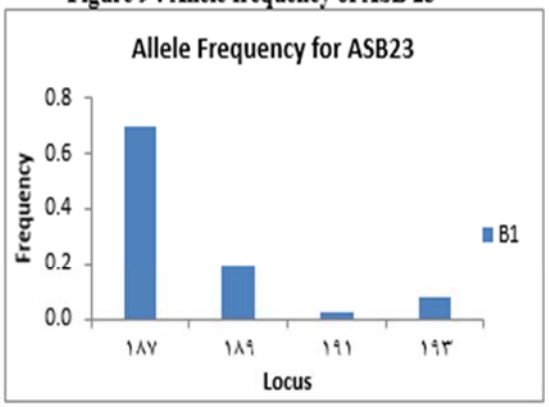


Figure6 : Allele frequency of ASB2

Figure 9 : Allele frequency of ASB 23



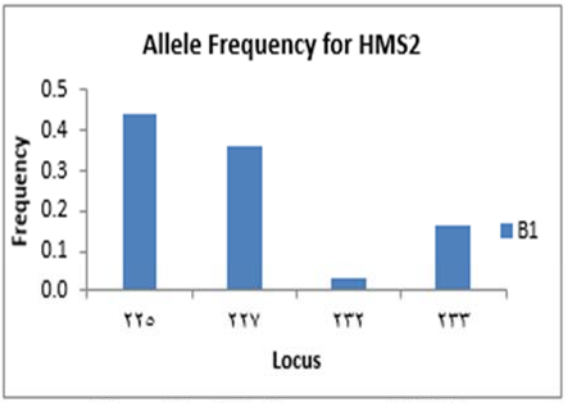
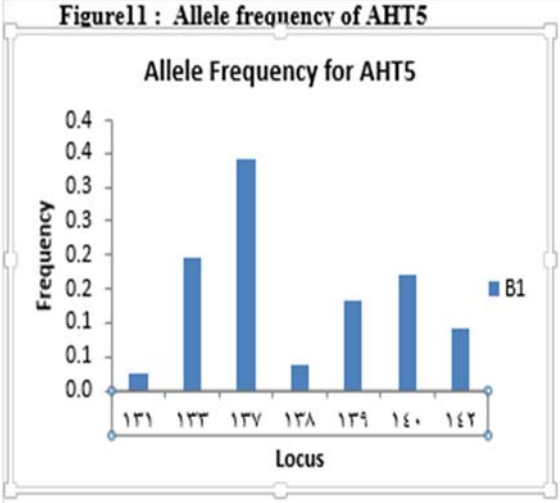


Figure10 : Allele frequency of HMS2

Figure11 : Allele frequency of AHT5



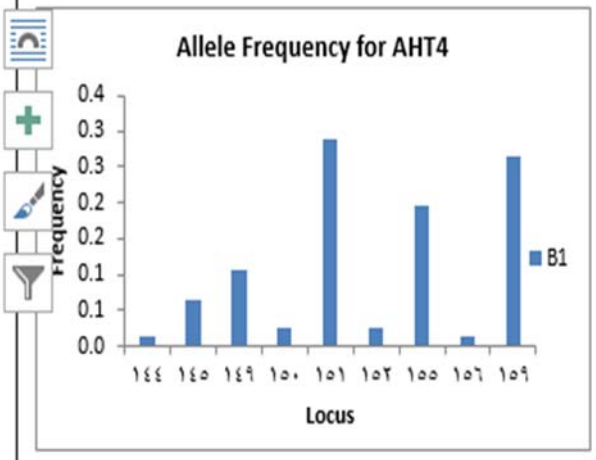


Figure12 : Allele frequency of AHT4

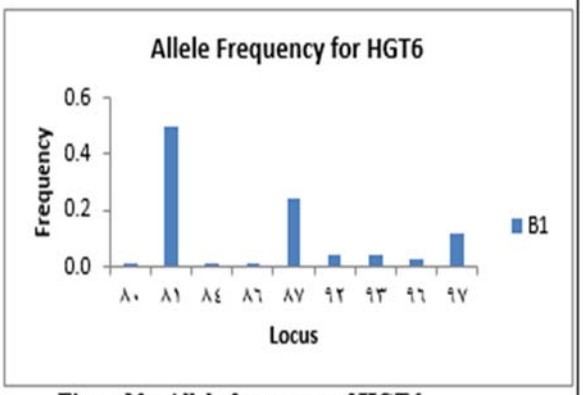


Figure13 : Allele frequency of HGT6

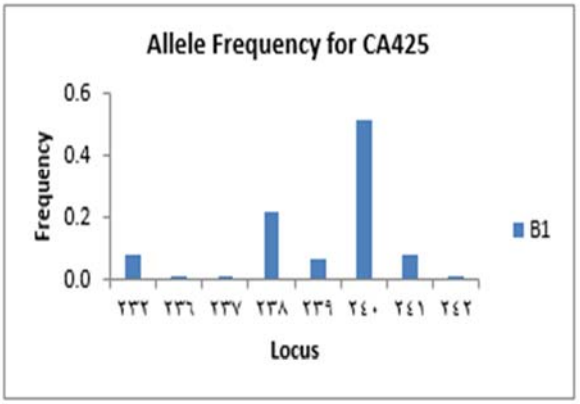


Figure14 : Allele frequency of CA425

Figure15 : Allele frequency of HMS1

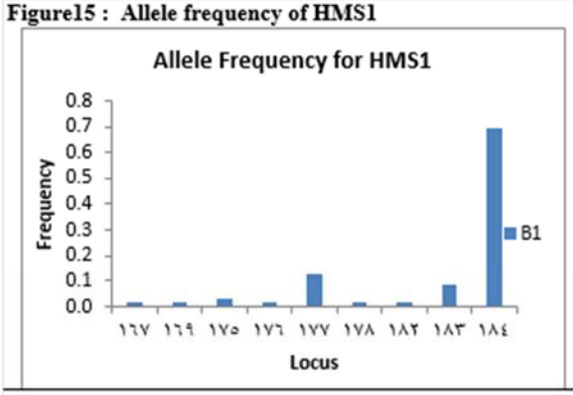
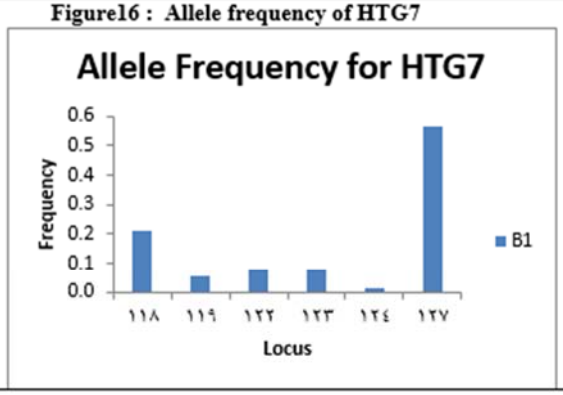


Figure16 : Allele frequency of HTG7



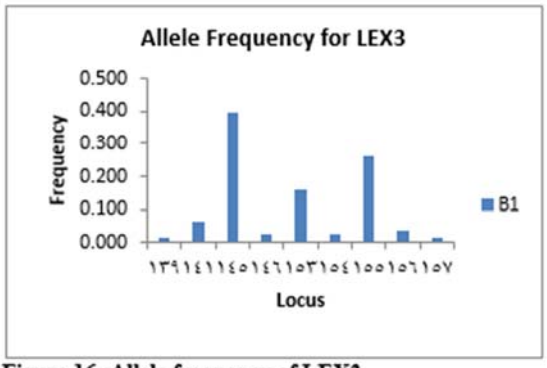


Figure 16 :Allele frequency of LEX3

There was 100 % matching between pedigree and DNA typing parentage for the 17 examined foals.

4. DISCUSSION:

The use of microsatellite typing for individual identification, parentage control and solving problems of questionable maternity or paternity is a routine procedure within the horse breeding industry in several countries (Kofler et al., 2008). Also, application of the DNA markers reveal extensive capability to distinguish among individual, and this ability has been utilized in analyses of reproductive success, kinship and parentage (Dimsoski, P.,2003).The aim of the present study was to construct a correct pedigree of Arabian horse family. After genotyping, parentage testing was performed according to Mendelism and ISAG guideline (Hill et al., 2002).The Polymorphism Information Content (PIC) is another measure of variation similar to heterozygosity and is calculated from allele frequencies. A high PIC value is indicative of a locus with high informativeness. In this study PIC value was > 0.8 which is very polymorphic. For linkage mapping Dierks selected markers with PIC values > 0.5 as markers with values below this level are insufficient for paternity testing. In their study, the average PIC value was 0.596 with a maximum of 0.866 (Dierks et al., 2007). Equine microsatellites were first characterized by (Ellegren et al. 1992) and (Marklund et al.1994) who isolated set of (CA) n repeats and demonstrated that they were highly polymorphic in horse. DNA based methods offer several potential advantages compared with conventional parentage testing systems because of their accuracy and specificity. Microsatellites have been chosen as the markers of choice because of their high levels of polymorphisms, which can be easily scored by a computer program. This indicates that DNA typing can be analyzed semiautomatically, alleles of the microsatellites were correctly inherited to

the next generation (Litt and Luty 1989).The Horse Genetic Committee of ISAG presented 12microsatellite markers (AHT4, AHT5, ASB2, ASB17, ASB23, HMS2, HMS3, HMS6, HMS7, HTG4, HTG10 and VHL20) as international minimum standard microsatellite marker system, as well as additional markers (HTG6, CA425, HMS1, LEX3, LEX33 and) to be typed for horse parentage testing as demonstrated in the example, seventeen foals were qualified by the compatibility of 17 markers according to Mendelian fashion in the present DNA typing for parentage verification (Myres et al., 2009). Our result was in agreement that microsatellite DNA typing could be useful for parentage testing. In conclusion, the 17 microsatellite markers system is theoretically considered to be greatly useful for parentage verification on Arabian horse in Egypt.

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اختبار البنوة للخيول العربية بمصر باستخدام تحليل الميكروستلايت للحامض النووي

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الملخص العربي

في هذه الدراسة تم تأسيس نظام اختبار البنوة للخيول العربية في مصر. العدد الكلي هو ثمانية وثلاثون عينه شامله على سبعة عشر مهر وخمسة طلايق وسته عشر فرسات. تم استخلاص الحامض النووي من جذور الشعر و تحليلها باستخدام تفاعل انزيم البلمرة المتسلسل المتعدد لبعض الاليلات المحددة في الخيول باستخدام سبعه عشر من واسمات الميكروستلايت معلمه بصبغات مختلفة .و تم اختبار هذه الواسمات طبقا لشروط المنظمة الدولية لوراثه الحيوان والتي توصي باستخدام ما لا يقل عن اثنتي عشر من هذه الواسمات و هم (VHL20, HTG4, AHT4, HMS7, HTG6, HMS6, HTG7, ASB17, LEX3) بالإضافة الي خمسه واسمات اخري وهم (HMS3, AHT5, ASB2, HTG10, and HMS2) وتم عمل اختبار تحليل لقطعه الحامض النووي المعلم عن طريق جهاز التحليل الجيني.واثبتت النتائج تحسن كبير للتعرف علي بنوة الخيول العربية عن طريق تحليل الميكروستلايت للحامض النووي بدقه عالية و مجهود اقل.

(مجلة بنها للعلوم الطبية البيطرية: عدد 27(1):100- 108, سبتمبر 2014)