



Classification of Saudi Arabian camel (*Camelus dromedarius*) subtypes based on RAPD technique

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Abstract

The genetic diversity and relationships amongst the dromedary (*Camelus dromedarius*) populations are poorly documented. This study compares, for the first time, variation in DNA fingerprinting of Saudi Arabian camels. The objective of this study was to analyze the inter- and intra-specific variation and genetic relationship between six widely distributed subtypes belonging to three types (Magateer, Magaheem and Beedh) of Saudi camels. One hundred twenty samples were analyzed by random amplified polymorphic DNA (RAPD) methodology using twenty universal decamer primers. All primers produced novel and polymorphic DNA fragments in all tested samples. The analysis of the electrophoretic patterns revealed a high polymorphism in size, number and intensity of bands. The generated fingerprint patterns were specific, i.e., one type could be differentiated from another. There are differences between the dendrograms generated from each subtype and the relationship between the other subtypes analyzed. However, the fingerprint profiles of the subtype individuals were virtually identical enabling easy distinction of the subtype. Estimation of genetic relationships between the 120 samples of 6 subtypes of 3 camel types using cluster analysis of the UPGMA method revealed two main clusters. Cluster A consisted of two subtypes (Magaheem A and B) with 0.76-0.85 similarity matrix. Cluster B consisted of three subtypes (Magateer B, Beedh A and B) with 0.76-0.83 similarity range. Cluster B is subdivided into two subgroups; Subgroup A includes Magateer B, Subgroup B includes Beedh A and B. Magateer Subtype A is 73% genetically similar to the rest of the subtypes. The average similarity among the twenty samples is more than 80%. Our results suggest a closer relationship between Beedh A and B; Magaheem A and B; and Magateer B and Beedh A and B. Magateer A is the least related to the other subtypes. The intra-specific analysis of the RAPD patterns showed a rich polymorphism in the heterogeneous subtypes of Magateer B, which is in concordance with the variability observed with other phenotypic markers. On the contrary, the other subtypes of Magateer A, Magaheem A and B, and Beedh A and B exhibit a homogeneous pattern indicative of a very low level of DNA polymorphism, which is congruent with the reduced variability found in these subtypes with other molecular markers. It is concluded that, the grouping indicated by the trees are reasonably well correlated with and supported the conventional morphological and physiological classification criteria. Identification of intra-specific variation suggests that RAPD could be having a potential to aid in identification and classification of Saudi camels. The reproducibility of the polymorphisms generated by RAPD in camel may lead to the development of subtype-specific DNA markers of native camel types in Saudi Arabia.

Key words: Classification, DNA fingerprinting, Saudi camels, molecular markers, random primers, phylogenetic relationships.

Introduction

Camels are in the taxonomic order Artiodactyla (even toed ungulates), sub-order Tylopoda (pad-footed), and family Camelidae. This family of mammalian animals is comparatively small¹. There are two genera within this family, *Camelus* (Old World camels) and *Lama* (New World camels). The genus *Camelus* consists of *Camelus dromedarius*, dromedary camel (one hump) and *C. bactrianus*, Bactrian camel (two humps)². All of the family members have great water efficiency, long necks, two toes with a web connecting them, and well-padded feet. Camels have several unique features. They walk on pads not hoofs. They do not have horns or antlers, and have high water retention ability, which helps their conserving body water. Moreover, camels are pseudoruminants; their stomach consists of 3 compartments, unlike other Artiodactyla ruminants such as cattle, sheep and goats³. Camel red blood cells are elliptical in shape such like those of chicken but with no nucleus⁴.

The dromedary, or one-humped camel, was described by Linnaeus in 1758⁵. There are no true wild dromedaries in the world today - they were domesticated between 4,000 and 2,000 B.C.E. for travel, meat and milk, and running, and became extinct in the wild around 2000 years ago. A large feral population [estimated between 25,000-80,000 individuals] exists in the Australian outback, descended from pack animals imported between 1840 and 1907. Dromedaries were also introduced into the southwestern United States in the middle of the 19th century for both transportation and meat, but due to the building of the railroads, the experiment was declared useless⁶. A further migration to the Southwest introduced populations of the smaller dromedary camel to the Saudi-Arabian Peninsula, Iran, Pakistan and part of Northern India. It is thought that camels might first have reached North Africa by human intervention, as they were not recorded in Egypt at the time of the Pharaohs. There are several million Arabian camels,

and most of them live with the desert people of Africa and Asia. All of the camelids have the same diploid chromosome number [2n=74]⁷. According to Abdo⁸, the diploid chromosome number of the Arabian camel was 74 chromosomes. There were 36 pairs of autosomes and one pair of sex chromosomes. The autosomes were also divided into three distinct groups; 31 pairs of acrocentric, four pairs of metacentric and one pair of submetacentric chromosomes.

In virtually all domesticated animals, differentiation into breeds and types takes place soon after domestication to select animals for certain traits such as morphological characters, particular size, shape, colour, behaviour and productivity. In case of camel, there have been relatively few changes. Saudi camels comprise 16% of the animal biomass⁹. Despite their differences, the camels do have their shape in common. These differences are among breeds or within breeds. No clear classification for camels exists but generally they can be classified according to habitat, colour and function³. According to the latter, breeders have classified camel into riding or baggage types. The former is a light finely built animal with small head and hump, large eyes, narrow face and thin supple skin, while the latter differs in size and configuration being heavily built with a large head, hump and feet, ample thorax and short neck^{10,11}. Several other methods such as psychological and reproductive criteria¹²⁻¹⁴ have been reported for differentiation of camel types. Each of these methods has its advantages and disadvantages.

Camel breeds vary in size, body conformation and color. The color is the most common character used for classification of camel breeds. Some are dark black and others have white or brown colors. Based on their colors, three main breeds of Saudi camels were distinguished, namely black (Magaheem), white (Magateer) and brown (Al Homr and Al Sofr). Magaheem camels are characterized by heavy weight, high meat production and slow motion. They reach 300 kg at one year of age and about 750 kg when five years old. They are found in the Middle and East areas of Saudi Arabia¹⁵. Magateer camels are medium to large animals and have pure white color. They are known to be good dairy camels and people liked them and have been proud of owing them through history. They produce about 3500 kg of consumable milk per year. They vary much in milk production within the group¹⁶. They reach 250 kg at one year of age and about 550 kg at maturity. They are common in the middle, north and south areas of Saudi Arabia^{15,17}. Brown-colored camels (Al Homr and Al Sofr) tend to be smallest in size with low milk production. Fully-grown camels can weigh up to 450 kg. They are medium to large animals and have pure white color. They vary much in milk production within the group. They are found in east and north areas of Saudi Arabia^{15,16}.

Very little is known about the biochemical and molecular phylogenetic relationships of the Arabian camel. Broad changes in the classification of the Arabian camels may result from molecular taxonomic studies. Although traditional phenotypic criteria are useful, the older classification techniques must be argued with this new taxonomic knowledge.

There is relatively limited information available on genetic diversity in camels. Studies of genetic variation among camels using protein electrophoresis revealed little or no genetic polymorphism^{18,19}. Recently, DNA markers have been exploited for species identification due to its stability at high temperatures

and its structure being conserved within all tissues of an individual. This has resulted in the development of species specific DNA probes²⁰ by random amplified polymorphic DNA (RAPD) and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) using polymerase chain reaction (PCR)²¹. PCR-based markers have been developed using either arbitrary or specifically designed primers from DNA repetitive sequences²². PCR-based RAPD marker techniques have been employed extensively for confirming genotypes of organisms at the level of both species and population²³. These markers have been successfully used in species identification in plants, bacteria and animals. The technique has been successful in providing new valuable information about the genetic structure and variation of different populations including mice²⁴, aspen strains²⁵ and buffalo grass²⁶. They are also used for the analysis of breeds and products in most animals that include fish, sheep and cattle^{22,27}. To our knowledge, there have been no reports of attempts to establish DNA fingerprinting-techniques in old world camelids, although it has been reported in new world camelids²⁸. Development of techniques for DNA fingerprinting for camelids, such as RAPD, AFLP and AP-PCR has been slow in comparison to that for other livestock species. DNA analysis techniques appear to be more powerful in detecting genetic variation among camels^{19,28} and racing camels²⁹.

The objective of the current study was to establish the foundation for selection of Saudi camels on the basis of their DNA fingerprinting. It aims also to develop and explore the potential application of RAPD markers in assessment of genetic diversity and/or similarity among the subtypes of Saudi camel types. Such system of classification will fit the requirements for the development of camel production and the improvement of the standard of their herders.

Materials and Methods

Animal material: Blood samples of one hundred twenty different individual camels from six subtypes belonging to three Saudi camel types (Magateer, Magaheem and Beedh) were collected from the Camel Research Center, King Faisal University, Al-Ahsa. They were obtained from the jugular vein of camels using 7-ml Vacutainers (Becton Dickinson) containing ethylenediamine-tetraacetate (EDTA) as anticoagulant.

DNA extraction and quantitation: Genomic DNA was extracted from blood samples using DNA Isolation Kit for Mammalian Blood (Roche Diagnostics, GmbH, Mannheim, Germany). All chemicals and reagents used in this study were of molecular biology grade. After purification, the resultant DNA was quantified using GeneQuant II spectrophotometer (Pharmacia Biotech), and its integrity was determined by agarose mini-gel electrophoresis.

Primers and RAPD assay: Random Amplified Polymorphic DNA (RAPD) analysis was performed by using 10-base primers: OPA (-03, -04, -08, -09, -11, -12 and -16), OPB (-02, -04, -05, -06, -07, -08, -10, -14, -15, -16 and -18), OPC-04 and OPF-05. The designations and sequences of these reproducible primers are given in Table 1. These primers were screened in one single program for suitability on the sampled representative subtypes from each type. Reproducibility was tested by performing PCR twice using two PCR machines (DNA Thermal Cycler 480; Perkin Elmer and Techne

Thermal Cycler; Progene, Cambridge, UK) in two different labs for Biotechnology at KACST.

Amplifications were performed in a final volume of 25 µl reaction mixture containing 15 ng of DNA, 50 pmol of primer, 1 U of *Taq* DNA polymerase (Roche Diagnostics GmbH), 100 µM of each dNTP (DNA polymerization mix, Pharmacia) and 1X PCR buffer. For RAPD-PCR the amplification program was as follows: an initial cycle at 95°C for 5 min; then 45 cycles of 94°C for 1 min, 36°C for 1 min and 72°C for 1 min, using a ramp of 30°C/min and a final step at 72°C for 5 min. To reduce the possibility of cross contamination and variation in the amplification reactions, master-mixes of the reaction constituents were always used. Standardization between enzyme batches and experiments were ensured by including standard controls in each: first control consisted of reaction mixture excluding DNA whilst second one was reaction mixture excluding any enzyme and/or primer.

The RAPD amplicons were separated by electrophoresis on 1.4% (w/v) agarose gels containing ethidium bromide (0.5 mg/ml) in 0.5 TBE. Electrophoresis took place for about 4 h at 80 V. DNA fragments were visualized by UV-transilluminator and documented using Gel Doc System 2000 (Bio Rad).

Table 1. The codes, sequences and GC% of the used reproducible primers, total number of amplified fragments and polymorphic bands generated in six subtypes of three Saudi Arabian camel types (Magaheem, Magateer and Beedh subtypes A and B, respectively) using these primers.

Primer	Sequence 5'→3'	GC%	Total amplified fragments	Polymorphic fragments
OPA-03	AGTCAGCCAC	60	110	32
OPA-04	AATCGGGCTG	60	107	22
OPA-08	GTGACGTAGG	60	98	34
OPA-09	GGGTAACGCC	70	75	33
OPA-11	CAATCGCCGT	60	90	22
OPA-12	TCGGCGATAG	60	73	18
OPA-16	AGCCAGCGAA	60	116	39
OPB-02	TGATCCCTGG	60	94	41
OPB-04	GGA CTGGAGT	60	64	60
OPB-05	TGCGCCCTTC	70	101	43
OPB-06	TGCTCTGCC	70	98	30
OPB-07	GGTGACGCAG	70	118	51
OPB-08	GTCCACACGG	70	110	44
OPB-10	CTGCTGGGAC	70	88	28
OPB-14	TCCGCTCTGG	70	86	33
OPB-15	GGAGGGTGTT	60	97	34
OPB-16	TTGCCC GGA	60	76	36
OPB-18	CCACAGCAGT	60	78	42
OPC-04	CCGCATCTAC	60	119	64
OPF-05	CCGAATTCCC	60	118	68
Total			1916	774

Data analysis: Comparisons of DNA profiles generated from each experiment were performed by Bio-Rad data base software. Fingerprint similarities values were based on the presence or absence of bands. Data were then computed and subjected to statistical analysis with computer software, Diversity Database Fingerprinting Software, to produce a genetic distance matrix using the formula of Nei and Li³⁰ which assesses the similarity between any two populations on the basis of the number of generated bands. The matrix was then computed to produce tree file using the similarity coefficient and unweighted pair group method with arithmetic averages (UPGMA) for cluster analysis³¹.

Results and Discussion

The present study was conducted for the identification and fingerprinting of camel types and subtypes of Saudi Arabia using RAPD analysis. High molecular weight DNA was successfully extracted from all samples. The ratio of A_{260}/A_{280} ranged from 1.6 to 1.84, which was sufficiently pure for use in PCR. PCR fingerprinting methods using repetitive sequences are based on the use of primers complementary to naturally occurring, highly conserved, repetitive DNA motifs, present in multiple copies in genomes of animals, plants and microbes^{32,33}. RAPD is a molecular technique which is easier, faster and cheaper than other DNA-based techniques. The potential of the RAPD technique, using the universal Operon primers for discrimination amongst the most common Saudi camel subtypes has been demonstrated.

A total of twenty primers with varying GC contents (13 sequences 60% and 7 sequences 70%) were screened. Only one PCR program was used to standardize the evaluation of the primers. All primers gave clear reproducible amplified polymorphic DNA products using two different thermal cyclers. To confirm that the observed bands were amplified genomic DNA and not primer artifacts³⁴, the exact PCR procedures were carried out as a replicate for each sample used for all primers. The highly reproducible RAPD fragments in this study perhaps were enhanced by winding the DNA's on a glass rod instead of collection by centrifugation³⁵.

The polymorphism came from different genomic DNA templates in each animal. The individual fingerprints from Saudi camels obtained by PCR amplification by the primer of OPA-04 are shown in Fig. 1. Several major bands in the fingerprints of every animal could be found. There were some minor bands in many lanes of the individuals tested. This indicates that the genetic material from the same type of animals was similar, although not entirely homogeneous.

RAPD profiles generated by the selected primers resulted in relatively simple DNA fingerprintings from which the subtype origin can be visually inferred, making this technique especially suitable for routine analysis. All primers produced novel and

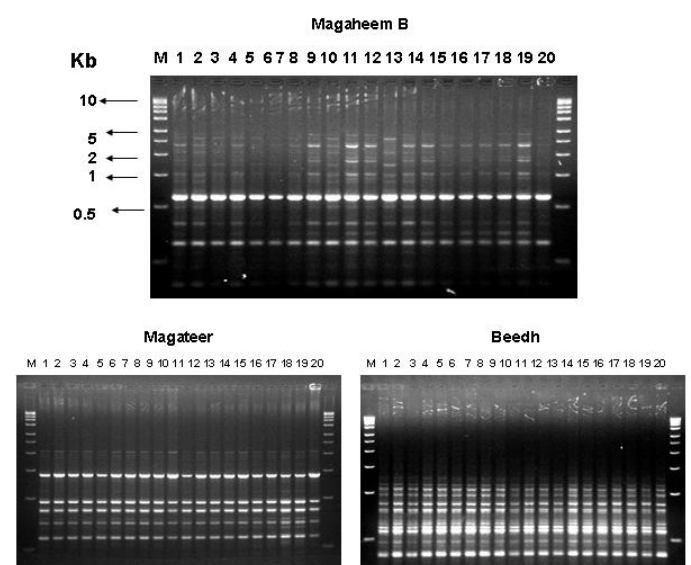


Figure 1. RAPD fingerprints detected in different individuals (Lanes 1-20) of three Saudi Arabian camel types (Magaheem, Magateer and Beedh types, respectively) using OPA-04 decamer primer. Lane M: Standard DNA molecular size marker.

polymorphic DNA fragments in all tested samples. There are differences between the dendrograms generated from each type and the relationship between the other subtypes analyzed. However, the fingerprint profiles of the subtype individuals were virtually identical enabling easy distinction of the subtype. Primers that revealed polymorphic products and were consistently and unambiguously scorable were: OPA-03, -04, -08, -09, -11 and 12, OPB-02, -05, -07, -08, -10, -14, -16 and -18, OPC-04 and OPF-05 (Fig. 2, Table 1). Out of 1916, a total of 774 reproducible polymorphic bands were obtained and scored as RAPD markers. The maximum number of polymorphic fragments (68) was recorded using primer OPF-05, while the minimum number (19) was scored using primer OPA-12. The maximum number of bands (119) was recorded using primer OPC-04, while the minimum number (64) was scored using primer OPB-04. Generally, experiments with the 70% GC primers showed clear fingerprint patterns, compared to the other primers. Five out of the seven 70% GC series showed fingerprint patterns that could be used to discriminate among several types, although not all of the types.

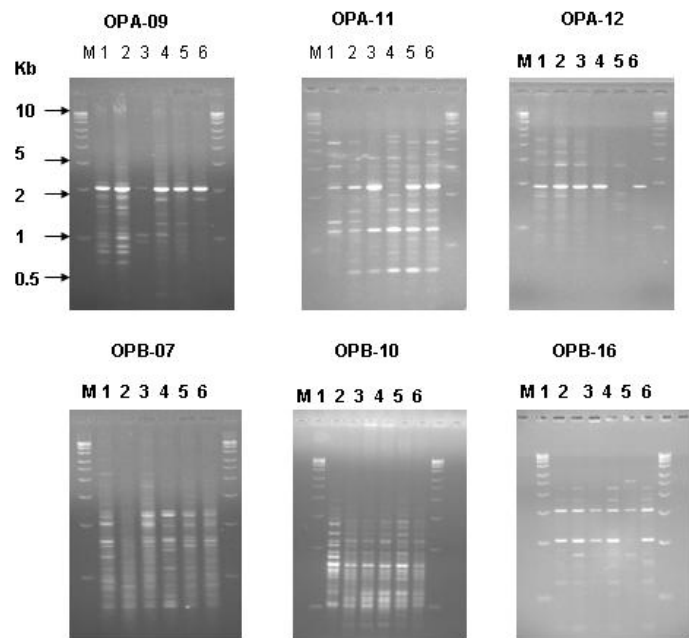


Figure 2. RAPD fingerprints detected in six subtypes of three Saudi Arabian camel types {Lanes 1-6 representing: Magaheem (Lanes: 1-2), Magateer (Lanes: 3-4) and Beedh (Lanes: 5-6) Subtypes A and B, respectively} using six decamer primers (OPA-09, -11 and -12, OPB-7, -10 and 16). Lane M: Standard DNA molecular size marker.

The RAPD showed enough genetic variation between and within dromedary types. Reproducible polymorphic bands with varying frequencies among the one hundred twenty camel samples were obtained with all primers used. Some DNA bands exhibited probable specificity for subtypes, whereas some other bands were observed to be useful in distinguishing the camel types. Some common bands were recorded as monomorphic (species specific). The highest number (85) was scored using primer OPA-04, while the lowest number (4) was recorded using primer OPB-04 (Table 1). The choice of primer to be used can greatly affect the amount of polymorphism generated. The number of known microsatellite loci is very much limited in camel as compared to human and mouse, but they are enough for molecular

characterization and give low number of polymorphic bands³⁶. These results are in agreement with that of Sherief and Alhadrami²⁹, who reported that different RAPD fragment patterns were obtained and found effective for detection of genetic variation in racing camels using seven Operon primers.

The similarity of the profiles of the different subtypes using both primers, OPC-04 and OPF-05, indicated their similar matching sites. Overall, RAPD fingerprintings obtained with the primers OPA-09, -11 and -12, OPB-07, -10 and -16 (Fig. 2) were clear enough to allow discrimination between the different subtypes. The reproducibility and simplicity of these patterns integrated by a small but reproducible number of bands makes the technique especially suitable for routine analysis due to easy interpretation of the results by visual inspection.

The similarity matrix between camel subtypes shows an average genetic distance range from 0.73 to 0.92 with a mean of 0.82. Thus, the camel types tested in this study are not highly divergent (mean > 0.6) at the DNA level. The smallest distance value was observed between samples of Subtypes Beedh A and B (0.92) which appear to be the most similar subtypes and can be closely regrouped. It is noteworthy that Beedh Subtypes A and B presented very limited average distance ranges (from 0.76 to 0.92) with the other subtypes except with Magateer Subtype B. Close genetic relationship between Subtypes Magaheem A and B was also observed. Magateer subtypes are characterized by a slight divergence at the DNA level and could be regrouped with the other subtypes.

The genetic distance matrix using the UPGMA algorithm was computed to cluster the data and to draw the precise relationships among the twenty tested camels. The resultant dendrogram shown in Fig. 3 illustrates the divergence between the used subtypes and suggests their tree branching. Estimation of genetic relationships between the 120 samples of 6 subtypes of 3 camel types using cluster analysis of the UPGMA method revealed two main clusters. Cluster A consisted of two subtypes (Magaheem A and B) with 0.76-0.85 similarity matrix. Cluster B consisted of three subtypes (Magateer B, Beedh A and B) with 0.76-0.83 similarity range. Cluster B is subdivided into two subgroups; Subgroup A includes Magateer B, Subgroup B includes Beedh A and B. Magateer Subtype A is 73% genetically similar to the rest of the subtypes. The average similarity among the twenty samples is more than 80%. Our results suggest a closer relationship between Beedh A and B; Magaheem A and B; and Magateer B and Beedh A and B. Magateer A is the least related to the other subtypes. The intra-specific analysis of the RAPD patterns showed a rich polymorphism in the heterogeneous subtypes of Magateer B, which is in concordance with the variability observed with other phenotypic or genetic markers. On the contrary, the other subtypes of Magateer A, Magaheem A and B, and Beedh A and B exhibit a homogeneous pattern indicative of a very low level of DNA polymorphism, which is congruent with the reduced variability found in these subtypes. This indicates that they may have originated from a common ancestor followed by cross-breeding of different types and subtypes. Similar findings have been reported in racing camels by Sherief and Alhadrami²⁹.

The main advantage of RAPD is that the technique usually generates some products that can be seen as DNA fingerprints on gel electrophoresis. Low RAPD polymorphism and the lack of evident organization observed among the camel types could be

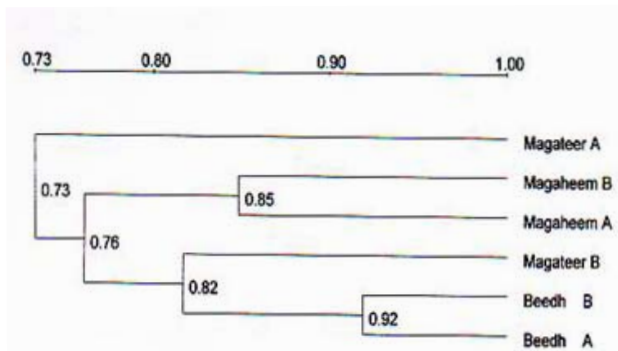


Figure 3. A dendrogram of phylogenetic relationships among six subtypes of three Saudi Arabian camel types (Magaheem, Magateer and Beedh Subtypes A and B, respectively) based on Nei and Li Similarity Coefficient obtained from twenty RAPD reproducible primers.

due to the nature of introduction of types in the country and the maintenance of germplasm in Saudi Arabia. Our results indicate that RAPD showed enough genetic variation between and within Saudi Arabian camel types and could be used for the discrimination of subtypes. RAPD is likely to be used as a rapid, qualitative way for subtype identification, and known standards must be run together each time a sample is tested. It is necessary to increase the number of types, subtypes and the number of primers to assess precisely the phylogenetic relationships of different camel types.

In addition, the grouping indicated by the phylogenetic trees were reasonably well correlated with the conventional criteria used for classifying types and subtypes of Saudi Arabian camels³. Although other methods can also be used, DNA fingerprinting of Arabian camel, because of its technical simplicity, excellent resolving power and high accuracy, seemed to be a good method for identifying these subtypes and recognizing phylogenetic relationships among them. It is concluded that, the grouping indicated by the trees are reasonably well correlated with and supported the conventional morphological and physiological classification criteria. These subtyping methods have the potential to aid in identifying camels. Identification of intra-specific variation suggests that RAPD could be having a potential to aid in identification and classification of Saudi camels. The reproducibility of the polymorphisms generated by RAPD in camel may lead to development of subtype-specific DNA markers of native camel types in Saudi Arabia. Overall, the assayed technique could be robust and simple method to be considered as an additional tool in Saudi camel identification.

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