

# Genetic Variants and Allele Frequencies of Kappa Casein in Egyptian Cattle and Buffalo Using PCR-RFLP

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## Abstract

Kappa casein (K-Ca) genetic variations affected quality and composition of the milk. Several variants of Kappa casein (K-Ca) gene locus IV have been reported with special interest for the 'B' allele for its relation to the milk protein and fat yields. Genotyping and allelic frequencies of K-Ca among Native Egyptian breeds of cattle and buffalo were the aim of the present study. PCR amplification of DNA isolated from 300 blood samples collected from Holstein and Baladi cattle and buffalo were performed followed by restriction fragment length polymorphism using *Hind-III* restriction endonuclease (PCR-RFLP). Detection of 'AA' and 'AB' genotypes in cattle breeds, 'BB' and 'AB' in buffalo and two alleles 'A' and 'B' in the studied breeds. Molecular selection for animals carrying the 'B' allele could impact breeding programs for dairy production in native cattle and buffalo breeds in Egypt.

**Keywords:** Kappa casein, polymorphism, cattle, buffalo PCR-RFLP

## 1. Introduction

Improvement of milk yield and its composition is the primary goal for animal selection in dairy industry. Animal selection on the basis of molecular markers considered to be more reliable than any other criterion (Riaz et al., 2008), as the using of DNA polymorphic markers allows the determination of individual genotypes at many loci and provides information on population parameters like allelic and genotypic frequencies which can be used as a tool for improving the animal selection through marker assisted selection (Kumar et al., 2006). Milk proteins polymorphism has attracted intensive research interest because its potential use as an aid to genetic selection of bovine breeds (Kemenes et al., 1999). Several studies have been reported that, milk protein variants, particularly caseins are associated with lactation performance and have major influence on milk composition and its processing properties (Kastonina et al., 2004; Denisenko & Kalashnikova, 2004; Konovalova et al., 2004). Polymorphism of casein gene has been intensely studied in different cattle breeds (Ceriotti et al., 2004; Wedholm et al., 2006). The association between K-Ca alleles and the total protein content, fat percentage and milk production was observed in several studies (Tsiaras et al., 2005, Verdier- Metz et al., 2001; Boettcher et al., 2004; Kubarsepp et al., 2005). The total size of k-Ca gene was about 13 KD (Alipanah et al., 2007) divided into 5 exons, with several genetic variants in different species. 'A' and 'B' alleles were the most common variants among it. K-Ca 'B' allele was reported to have a favorable and significant effect on both milk yield and milk protein content in Reggiana dairy local cattle (Caroli et al., 2004), different Indian buffaloes breeds (Raj et al., 2008), nili-ravi buffalo (Riaz et al., 2008). Also, its favorable effect on milk technological properties and cheese yield in taurine and zebu cattle breeds (Ceriotti et al., 2004) and Brazilian cattle (Azevedo et al., 2008), desirable coagulation properties reported in Finnish Ayrshire cows (Ikonen et al., 1997).

Medrano and Aguilar-Cordova (1990) identified a restriction fragment length polymorphism (RFLP) at the K-Ca bovine locus and detected two alleles 'A' and 'B' that were differed by two amino acid substitutions (Lin et al., 1992). Those K-Ca genotypes had highly significant effect on protein content such that 'BB' genotype was associated with high protein content than 'AA' genotype, also the cheese yield from cow with K-Ca 'BB' genotype was 10% higher than that of genotype 'AA' (Azevedo et al., 2008).

Using molecular markers allow direct genotyping for K-Ca; whose polymorphism has direct and potent effect on milk production, with accuracy to be used in dairy cattle improvement program.

The objective of the current work was to identify the genotypes and allelic frequencies of K-Ca locus in Egyptian cattle and buffalo breeds aiming to improve the milk yield and composition through genetic selection in Egypt and to highlight its importance to be adopted elsewhere in the world dairy program.

## 2. Material and Methods

### 2.1 Genomic DNA Isolation

A total of 300 blood samples were collected from local Baladi breed of dairy cows, Holstein cattle and buffalo from different farms in Egypt, unrelated individuals following the recommendations suggested by ISAG/FAO advisory group on animal genetic diversity (FAO, 1998). Blood samples were collected in K<sub>3</sub>-EDTA coated sterile vacuainers and stored at -20°C until used for genomic DNA extraction.

Genomic DNA was extracted from each blood sample according to Sambrook et al. (1989). The quality of the DNA was checked on 1% agarose gel.

### 2.2 PCR Amplification

A 379 bp fragment containing exonIV of K-Ca gene was amplified through PCR using forward K-F: 5'-CACGTCACCCACACCCACATTTATC-3' and reverse K-R: 5'-TAATTAGCCCATTTTCGCCTTCTCTGT-3' primers (Mitra et al., 1998). The PCR mixture composed of 5<sub>x</sub> PCR master mix (Jena BioScience™), 25 pmol from each primer, 1 µl from BSA, 50 ng of genomic DNA and sterilized distilled water to make a final volume of 25 µl. The PCR reaction included pre-denaturation for 5 min at 95°C followed by 35 cycles at 95°C for 1 min, 64°C for 90 sec, 72°C for 1 min and a final extension of 10 min at 72°C.

### 2.3 K-Ca Genotyping Using RFLP

The PCR products were digested with Hind-III restriction enzyme to discriminate the different allelic variant for K-Ca (Rottmann & Schlee, 1992). The restriction digestion was performed in a total volume of 25 µl (15 µl of PCR product, 2.5 µl enzyme buffers, 0.5 µl enzymes and 7 µl distilled water) and placed in the incubator at 37°C for 3 h. The restriction products were electrophoresed on 10% non-denaturing polyacrylamide gel and electrophoresed in 1 x TBE buffer (89 mM Tris (hydroxymethyl) aminomethane-boric acid and 2 mM EDTA) on a minislab gel at room temperature at 150 V for 5 min, then at 80V until the DNA bands migrated about 2/3 of the gel (Rachagani et al., 2006). The gels were stained by Etheduum bromide (1 mg/ml) for 2 min and then destained in water for 15 min.

### 2.4 Statistical Analysis

Direct counting was used to estimate genotyping and allele frequencies of K-Ca genetic variants. The chi-square test ( $\chi^2$ ) was used to check whether the populations were in Hardy-Weinberg equilibrium. Allele frequencies and mean expected heterozygosities per locus and population were calculated using Arlequin ver. 3.11 package programs (Excoffier & Heckel, 2006).

## 3. Results and Discussion

Early and precise identification of milk protein polymorphism have great impact on dairy cattle breeding strategies (Scheepers et al., 2010). Using the PCR-RFLP technique for identification the genetic polymorphism in K-Ca allowed both rapid and efficient determination of the genetic variation in this gene regardless the age and sex of animals, leading to establishment and increasing the frequency of desired alleles among our Egyptian animals. Genotypes and allelic frequencies distribution for K-Ca gene in Egyptian Holstein, Baladi cattle and buffalos breeds were detected. In both cattle breeds the 'AA' & 'AB' were detected with similar genetic frequencies and absence of 'BB' genotype with higher frequency of 'A' allele (0.63) in both breeds (Table 1). This is the first reported research on genotype and allele frequencies for K-Ca gene in native Egyptian Baladi cattle breed however, such reports are available for many other breeds like Anatolian Black and East Anatolian Red breeds of cattle (Gurses & Yuce, 2012).

Hardy-Weinberg proportions tests are frequently used to check on random mating in populations and the deviations from expectation are used to estimate inbreeding coefficients. Some events, such as an accumulation of some genotypes, subdivision of the population, mutation, selection, migration, or endogamy can result in a state of disequilibrium within the population (Tambasco et al., 2000; Vasconcellos et al., 2003). Both cattle breeds were deviated from equilibrium which may be due to migration or subdivision.

The same results were detected in Anatolian black and East Anatolian red native cattle breeds of Turkey (Gurses & Yuce, 2012). Red Pied (Alipanah et al., 2005), Black Pied cows (Doosti et al., 2011) and Romanian spotted cattle (Daniela et al., 2007). However, the 'A' allele frequency of the K-Ca gene of cattle identified in this study was still

lower compared to the reported figures of some other countries such as USA, Europe, Brazil and Japan which having a higher 'A' allele frequency at a range of 0.8-0.9 (Swaisgood, 1992; Rachagani & Gupta, 2008).

Table 1. The distribution of Kappa casein genotypes and allele frequencies in Holstein cattle, Baladi cattle and Buffalo in Egypt and Hardy-Weinberg equilibrium

Animal breed		Genotype			Allelic frequency	
		AA	BB	AB	A	B
Baladi cattle	Obs	26	Zero	74	0.63	0.37
	Exp	39.69	13.69	46.62		
	Genotype frequency	0.26	Zero	0.74		
	$\chi^2$	34.47		0.0001 <sup>***S</sup>		
Holstein cattle	Obs	24	Zero	76	0.62	0.38
	Exp	38.44	14.44	47.12		
	Genotype frequency	0.24	Zero	0.76		
	$\chi^2$	37.54		0.0001 <sup>***S</sup>		
Buffalo	Obs	Zero	75	25	0.125	0.875
	Exp	1.63	76.5	21.87		
	Genotype frequency	Zero	0.75	0.25		
	$\chi^2$	2.099	propapablity 0.1473 <sup>NS</sup>			
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$\chi^2$  = chi-square value; Obs.: observed frequencies; Exp.: expected frequencies on the basis of Hardy-Weinberg law. \*\*\* Statistically significant, the population is in Hardy-Weinberg frequencies (is not rejected).

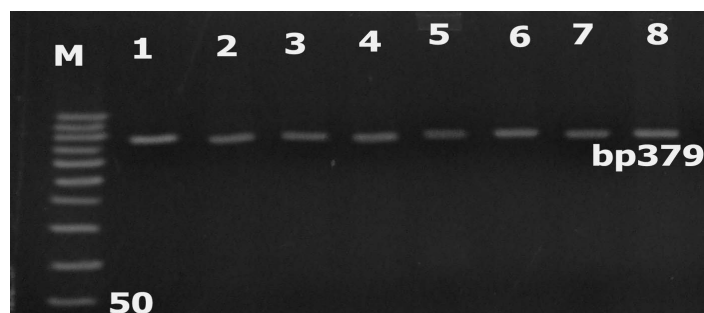
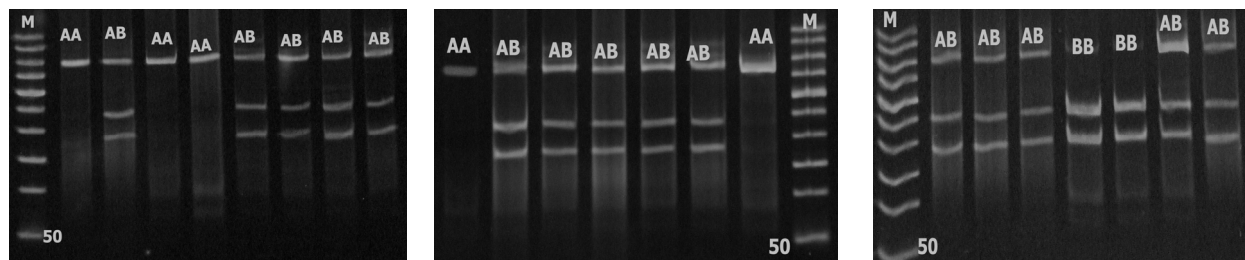


Figure 1. Electrophoresis of Kappa casein gene PCR products on 2% agarose gel

Agarose gel (2%) showing PCR products of Kappa casein of Baladi cattle (lanes 1-3), Holstien cattle (lanes 4&5), and buffalo (lanes 6-9). All lanes showing a single specific band with 379 bp expected size. Lane M represents the 50 bp DNA molecular weight marker (Jena BioScience™).



A. The RFLP patterns of the k-casein gene after digestion with hindIII on 10% polyacrylamide gel with 50 pb ladder as Molecular Marker in Holstein cattle.

B. The RFLP patterns of the k-casein gene after digestion with hindIII on 10% polyacrylamide gel with 50 pb ladder as Molecular Marker in Holstein cattle.

C. The RFLP patterns of the k-casein gene after digestion with hindIII on 10% polyacrylamide gel with 50 pb ladder as Molecular Marker in buffaloes .

Figure 2. Electrophoretic patterns of 379 bp PCR products of Kappa casein gene digested with Hind III endonuclease on 10% PAGE

On the other hand, Allmere et al. (1998) and Oner and Elmaci (2006) reported the presence of ‘A’ and ‘B’ alleles for K-Ca, with highest frequency ‘B’ allele in Holstein breed. Cows with ‘AB’ and ‘BB’ genotypes showed significant higher milk proteins and fat content when compared with that of ‘AA’ genotype (Botaro et al., 2009) suggested that selection of cows with ‘AA’ genotype for meat production is preferred.

Regarding Egyptian buffaloes, absence of ‘AA’ genotype of K-Ca genotype, detection of ‘BB’ and ‘AB’ genotypes with extremely higher frequency of ‘B’ allele (0.875) were observed (Table 1). These results are in agreement with Singh et al. (2005), Patel et al. (2007). However, Pipalia et al. (2001), Ontaviano et al. (2005) Riaz et al. (2008) and Ren et al. (2011) reported that the K-Ca in buffalo is monomorphic with only one allele ‘B’.

Buffalo were found to be in Hardy-Weinberg equilibrium (Table 1), suggested that the Kappa casein gene was not influenced by selection.

Many researchers reported that K-Ca genotypes especially ‘BB’ one was related to the quality of milk and cheese making (Ikonen et al., 1999; Patil et al., 2003). Therefore, the ‘BB’ genotype in Holstein cattle seems better suited for improvement of the quality of milk and cheese making in Egypt. This genotype was absent in our cattle breed so we have recommended here direction to increases frequency of ‘BB’ genotype through genetic selection of breeding bulls carrying ‘BB’ genotype through artificial insemination or direction of cattle breeds to meat production rather than milk production.

In conclusion K-Ca genetic polymorphism could be used as useful marker for genetic selection. Also our study can help in maintaining a high frequency of ‘B’ allele as the favorable one for increasing milk quality and quantity in our commercial cattle breeds.

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