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Detection of Caprine-specific Nucleic Acid Sequences in Goat Milk Using Polymerase Chain Reaction

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ABSTRACT

Introduction: This study was carried out to evaluate PCR-based method for detection of DNA in goat milk. It utilized primers targeting the mitochondrial cytochrome -b (mtcyt-b) gene, which was used as a target DNA for PCR amplification. **Methods:** For the specific identification of goat mtcyt-b gene, pair of primers (GSL1, GSR2), were used, which produced a 428 base pair (bp) PCR product from milk samples as well as from peripheral blood. Amplification products were visualized on ethidium bromide-stained agarose gels. **Results and Discussion:** Amplification products were not detected when the PCR was applied to DNA from animal species including cattle, sheep, swine, camel, deer, horse, donkey, and human, which indicates that the 2 pairs of primers are specific for goat. **Conclusion:** DNA can be extracted from goat milk and would be advantageous in the variety of application such as species identification in milk and milk.

Key words: Goat milk; DNA, Rapid detection, PCR assay.

1. INTRODUCTION

Collection of milk is a non invasive procedure. The technical convenience of milk as a source of DNA can be expected to increase the field of application of the marker-based methods for genetic analysis of goat genome. Recently, species identification of dairy products has received great attention. It has a remarkable importance for several reasons related to governmental regulation and public health. Mixture in dairy products and species substitution should be observed to be a cause of human adverse reactions (Bottero et al, 2003). The source of DNA in milk could be from somatic cells (Fahr et al, 1999). Recently DNA was detected from human milk (Sami et al, 2009).

Goat milk is similar to cow milk with around 87 percent water, 67 percent energy, 3.3 percent protein, 4.0 percent fat, and 4.6 percent carbohydrates (Belanger and Jerry, 2001). Goat milk differs from cow and human milk in several ways, among them higher digestibility and lower lactose (Larson, 1978). One difference is that goat milk has smaller fat globules present due to the lack of the enzyme that aggregates the globules in the milk (Belanger and Jerry, 2001). The objective of the present study was to evaluate milk as a source of goat DNA and as a substrate for PCR amplification using mitochondrial cytochrome-b gene as a target DNA for PCR amplification.

2. MATERIAL AND METHODS

2.1. Study design

This was prospective study in which samples are collected throughout the study

2.2. Study Area

This study was conducted at the Molecular Biology Laboratory, Faculty of Veterinary Medicine, University of Khartoum, and the national Ribat University, Khartoum, Sudan. During the period from February to July 2010

2.3. Study population

25 Nubian Goats, maintained at the dairy farm of the faculty of Veterinary medicine, University of Khartoum, Shmbat were included. All goats were in good health particularly with no clinical mastitis.

2.4. Data collections

Milk samples collection from goats: Before collection, the teats were cleaned with alcohol to avoid samples contamination from skin. Milk (2.5 ml) was collected in sterile 2.5ml centrifuge tube by hand milking, samples were stored at 4°C until testing for extraction of total genomic and mitochondrial cytochrome-b (mtcyt-b) DNA. The extracted DNA was used as a target DNA for PCR amplification.

Blood samples collection: Blood samples were collected for preparation of positive control, blood samples

were collected in clean sterile vacutainers, containing ethylene diamine tetra acetic acid (EDTA), from goats (positive controls), from the animals attended at the veterinary teaching hospital.

The blood samples then centrifuged in bench centrifuge (Hettich Zentrifugen, D-785320, Germany) in order to separate the buffy coat which is rich in white blood cells and used for extraction of total genomic and mitochondrial cytochrome-b (mtcyt-b) DNA. The extracted DNA was used as a target DNA for PCR amplification

DNA extraction from milk samples: Extraction of DNA from goat milk and peripheral blood was made possible using a commercially available QIAamp blood kit (QIAGEN Inc.Chatsworth, Canada) according to the manufacturer's instructions. In details, 200µl of milk samples, 20 µl of proteinase K enzyme stock solution, and 200 µl of lysing buffer (LA buffer) were pipetted into 1.5 ml eppendorf tube and the mixture was vortexed on the vortexing machine (Janke &Kunkel,GmbHu.CoKG, Germany) and incubated at 60°C for 10 minutes. 200µl of absolute ethanol were added to the sample and mixed by vortexing. The mixture then was transferred to the QIAamp spin column, and was placed in a clean 2ml collection tube and centrifuged in microcentrifuge (Hettich Zentrifugen, 12-24, Tuttlingen, Germany) at 8000 rpm for 1 minute. The QIAspin column was washed firstly with 500 µl of washing buffers 1 (AW1) at the same previously mentioned centrifugation speed and rewashed using washing buffer 2 (AW2) at speed 1200 rpm centrifugation speed for 3 minutes. The QIAamp spin column was then placed in a clean 1.5 ml eppendorf tube and the DNA was eluted with 200 µl of double distilled water preheated at 70°C. Maximum DNA yield will be obtained by spinning at 1200 rpm for 1 minute after remaining for 1 minute in the room temperature. The DNA concentration was determined by spectrophotometer at 260-wave length. Five microliters of the suspended nucleic acid will be used in the PCR amplification

Selection of goat primers for PCR amplification: For PCR amplification a pair of goat-specific primers (GSL1&GSR2) was designed from the caprine mtcyt-b gene sequences. GSL1 included bases 284-303 of the positive sense strand (5)- TCA TAC ATA TCG GAC GAC GT. whereas GSR2 included bases 693-712 of the complementary strand (5)- CAA GAA TTA GTA GCA TGG CG. Using of this pair of primers (GSL1&GSR2) in PCR assay resulted in amplification of a 428-bp PCR product from caprine mtcy-b DNA.

The primers were synthesized on a DNA synthesizer (Milliigen/Biosearch, a division of Millipore Burlington, MA) and purified using oligo-pak oligonucleotide purification columns (Glen Research Corporation, Sterling, VA) as per manufacturer's instructions.

2.5. Polymerase chain reaction (PCR): A stock buffered solution containing 250 µl 10X PCR buffer, 100 µl of MgCl₂, 12.5 µl of each dATP, dTTP, dGTP and dCTP was prepared in 1.5 ml eppendorf tube, and double distilled water was added to bring the volume of the stock buffer solution to 1.5 ml. the primers were used at a concentration of 20µmole/L which appears to 2 µl . Next, 5.0 µl of

the target DNA was added to 42 µl of the stock solution in 0.5 ml PCR tubes and mixed by vortexing . This is followed by 1.0 µl of Taq DNA polymerase (Perkin Elmer) which was used at a concentration of 2.5 units. All PCR amplification reactions were carried out in a final volume of 50 µl. The thermal cycling profiles were as follows: 2-minutes incubation at 95°C, followed by 40 cycles at 94°C for 1minute, 57°C for 30 sec and 72°C for 45 sec, and a final incubation at 72°C for 10 minutes. Thermal profiles will be performed on a Techne PHC-2 thermal cycler (Techne, Princeton, NJ,USA).

2.6. Visualization of PCR products

All PCR amplification product samples were visualized using agarose gel electrophoresis. The 10X Tris borate EDTA (TBE) buffer was diluted to 1X solution which was used to prepare 1.0 % agarose gels and as running buffer in electrophoresis after it was stained with ethidium bromide as 0.5µg/ml. 15 µl from each PCR reaction containing amplified product was loaded onto gels of 1.0 % agarose (FMC Bioproduct, Rockland ME) and was electrophoresed. The results were visualized under UV light transilluminator. The results were then photographed by gel documentation system.

3. RESULTS AND DISCUSSION

DNA was successfully extracted from goat milk samples used in this study. The DNA yield varies with the different stages of lactation as compared with the yield from peripheral blood leukocytes. The PCR-based assay described in this study afforded sensitive and specific identification of goat mtcyt-b DNA. Using the pair of primers (GSL1, GSR2). The sensitivity studies indicate that, the 428bp PCR products were detected from not < 1.0 pg of goat mtcyt-b gene (Fig.1). The specificity studies for goat primers indicate that the described PCR assay failed to amplify the specific PCR product from DNA extracted from other animal species including cattle, sheep, swine, camel, deer,

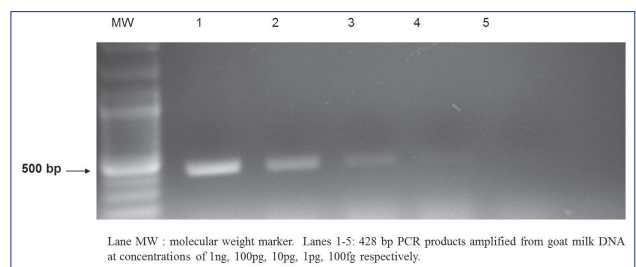


Figure 1.

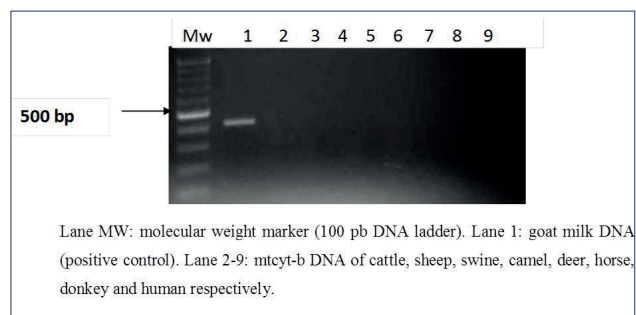


Figure 2.

horse, donkey, and human (Figure 2). Using primers GSL1, GSR2, amplification of the goat-specific 428 bp PCR product was produced from different milk samples collected from goats included in this study (Figure 3, 4, 5).

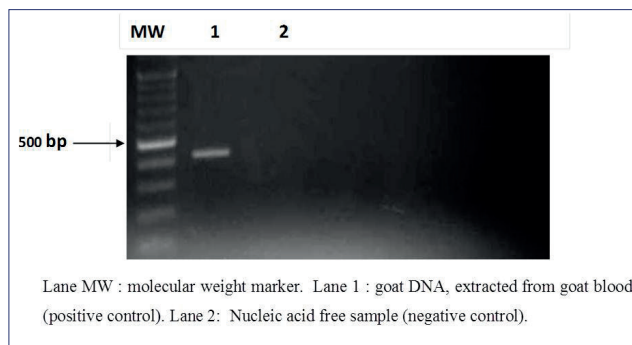


Figure 3.

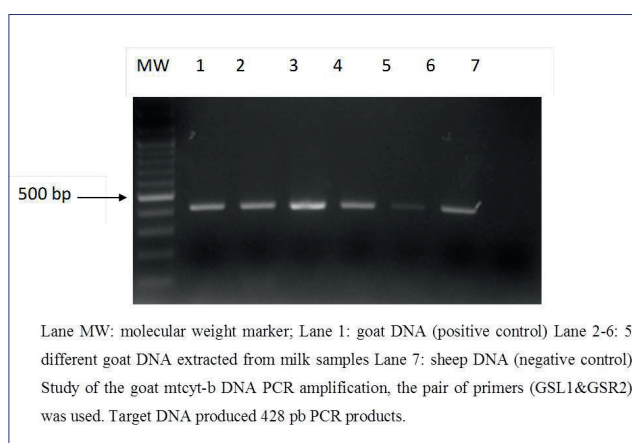


Figure 4.

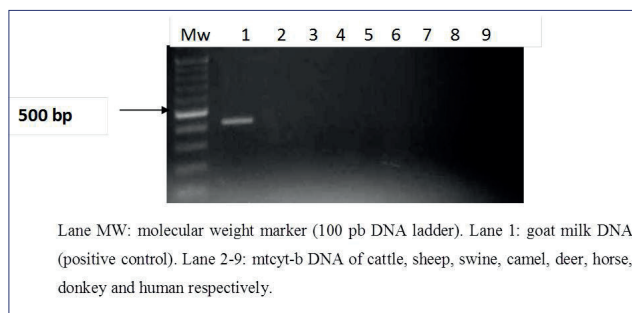


Figure 5.

In goat, peripheral blood leukocytes are the usual source of DNA for genotyping. However, obvious technical difficulties exist in the collection of blood samples from large numbers of individuals among widely separated herds, limiting the application of marker-based methods for genetic analysis and for genetic improvement of economic traits in dairy goat. The scientific data presented in this study indicate that DNA extracted from goat milk could serve as substrates for PCR amplification of the full length of the caprine mitochondrial cytochrome-b gene or a fragment of the gene. Since collection of milk is a non invasive procedure, it can often substitute for blood as a source of DNA. The technical convenience of milk as a source of DNA can be expected to increase the field of application of the marker-based methods for genetic analysis of goat genome.

Recently, species identification of dairy products has received great attention. It has a remarkable importance for several reasons related to governmental regulation and public health. Protection against species substitution or admixture in dairy products is of significant importance (Bottero et al, 2003). Milk is known to be frequent cause of food allergies. It was found that most milk proteins, even at low concentration, are potential allergens (Sampson, 2003). Also, cow's milk was reported as the main dairy product responsible for human adverse reaction (Rance et al, 2005). Thus, the counterfeiting of goat's milk with cow's milk may be considered as a health risk making Species identification an important issue in current food safety requirement. The common fraudulent practice found in the dairy production line is the use of a cheaper type of milk in substitution of more expensive ones. Currently, different methods are used for species identification in milk and milk products including immunological (Addeo et al, 1995), electrophoretic (Cartoni et al, 1998), and chromatographic techniques (Pellegrino et al, 1991). Among these methods, capillary electrophoresis, two dimensional electrophoresis, isoelectric focusing of milk caseins which is the European Community reference method for cow's milk detection (ECR, 1996), also ELISA are reported (Molina et al 1999). However these methods can't always distinguish milk from closely related species and not suitable for heat treated milk. The PCR amplification technology, described in this study, provides a simple, rapid, reliable and sensitive method for species identification and differentiation. The time required for the PCR amplification was approximately 3 hours; this means that confirmatory diagnosis could be obtained within the same working day.

E. Lipkin et al. (1993) reported that milk is less reliable source of DNA than is blood because it requires large size of sample and high concentration of somatic cells (E. Lipkin et al, 1993). But in our study we used only 200µl of milk sample for extraction of DNA, and the PCR products were the same in length and approximate quantity for milk, for DNA extracted from milk, and for DNA extracted from blood, this difference may reflect the detection procedures described above.

The intensity of DNA signal in gel electrophoresis, show that, there is a decline in the DNA content in the late lactation periods. This may indicate the milk DNA content is high in the first and second lactation periods and then declines. For more significant results DNA quantification by spectrophotometer is required which provide information about DNA concentration through lactation periods.

In this study PCR-based assay use DNA targets in the mitochondrial genome. These non-nuclear targets possess several advantages over nuclear genes (Unsel et al,1995). They are generally more abundant in any given sample than single-copy nuclear genes, and, because mitochondrial DNA has a relatively high mutation rate compared with the nuclear DNA, they contain a greater accumulation of point mutations that can be used to better define species differences. Moreover, mitochondrial DNA tends to be inherited through the maternal germ line, and the resulting lack of heterozygosity in the alleles under study simplifies analysis (Kocher et al, 1989). The mtcyt-b DNA

was selected in this work as the target sequence for species identification.

4. CONCLUSION

PCR-based assay described in this study would be advantageous in the variety of conditions including comparative genomics, species identification in milk and milk products, experimental physiology and can be recommended in the quality control departments in order to support policies and regulation of import/export of milk and milk products.

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REFERENCES

1. Addeo FMA, Nicolai L, Chianese L, Moio S, Musso S, Bocca A, Del Giovine A. A control method to detect bovine milk in ewe and water buffalo cheese using immunoblotting. *Milchwissenschaft*. 1995; 50: 83-85.
2. Belanger J. *Storey's Guide to Raising Dairy Goats*. North Adams, MA: Storey Publishing, 2001.
3. Bottero MT, Civera T, Nucera D, Rosati S, Sacchi P, Turi MM. A multiplex polymerase chain reaction for the identification of cow's, goat's and sheep's milk in dairy products *International Dairy J*. 2003; 13: 277-282.
4. Cartoni GP, Coccioli F, Jasionowska R, Masci M. Determination of cow milk in buffalo milk and Mozzarella cheese by capillary electrophoresis of the whey protein fractions. *Italian J Food Sci*. 1998; 2: 127-131.
5. Lipkin E, Shal Om A, Khatib H, Soller M, Friedmann A. Milk as a Source of Deoxyribonucleic Acid and as a Substrate for the Polymerase Chain Reaction. *J Dairy Sci*. 1993; 76: 2025-2032.
6. ECR, European Commission Regulations. No. 1081/96 Reference method for the detection of cow's milk and cow's milk casein in cheese made from ewes, goats and buffalo milk or mixtures of ewes, goats and buffalo milk. *Official Journal of the European Commission*. 1996; L142: 15-25.
7. Fahr RD, Schulz J, Finn G, von Lengerken G, Walther R. Cells count and differential cell count in goat milk-variability and influencing factors. *Tierarztl Prax*. 1999; 27: 99-106.
8. Kocher TD, Thomas WK, Mayer A, Edwards SV, Pääbo S, Villablanca FX, Wilson AC. Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. *Proc Natl Acad Sci*. 1989; 86: 6196-6200.
9. Larson B. *The Dairy Goat as a Model in Lactation Studies*. *Dairy Science Journal* 1978; 61: 1023-1029.
10. Molina L, Martin-Alvarez PJ, Ramos M. Analysis of cows', ewes' and goats' milk mixtures by capillary electrophoresis: Quantification by multivariate regression analysis. *International Dairy J*. 1999; 9: 99-105.
11. Pellegrino L, De Noni I, Tirelli A, Resmini P. Detection of bovine milk in cheese from minor species by HPLC of whey proteins. Note 1-application to water buffalo Mozzarella cheeses. *Sci Tec Latt-Cas*. 1991; 42: 87-101.
12. Rance F, Grandmottet X, Grandjean H. Prevalence and main characteristics of diagnosed with food allergies in France. *Clinical Experimental Allergy*. 2005; 35: 167-172.
13. Abdalla SF, Musa OA, Aradiab IE. Evaluation of milk as a source of human DNA in lactating women using PCR, *International journal of molecular medicine and advance sciences*. 2009; 5(1-4) : 6-9.
14. Sampson HA. Food allergy. *Journal of Allergy and Clinical Immunology*. 2003; 111: 540-547.
15. Unseld M, Beyermann D, Brandt P, Hiesel R. Identification of the species origin of highly processed meat products by mitochondrial DNA sequences. *PCR Methods Appl*. 1995; 4: 241-243.