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Polymerase Chain Reaction for Identification of Mitochondrial Cytochrome b Gene in *Gazella dorcas* in Sudan

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ABSTRACT

In this study two assays, PCR was developed for rapid detection of mcyt-b. Whole blood in EDTA were collected from 17 *Gazella dorcas* from Hilat Kuku Zoo, Elkadaru's farm and Mozamel Elkurdi's farm in East of the Nile, Khartoum State. The origins of these animals are Dongola, River Nile- Northern Sudan and Butana- Central Sudan. Other blood samples were collected in EDTA from Shambat-Bahry from sheep, goats, cows, camels, donkeys, horses and pigs. The blood samples were used for DNA extraction by using commercial kit (QIAamp blood kit- QIAGEN Inc Chatsworth, CA, USA). Other samples were also taken from a slaughtered gazelle from heart, lung, liver, kidney, spleen, tongue, muscles of scapula, intestine, rumen, reticulum, omasum and lymph nodes. The size of PCR product was 421 bp. For specificity of PCR, DNA extracted from (dorcas gazelles, cows, sheep, goats, camels, pigs, horses and donkeys) was used with GZ1 and GZ2 primers. The PCR line appeared only with the DNA extracted from dorcas gazelle. The product was sent for sequencing to Macrogen Company-World Meridian 10F, Gasan-dong, Geumcheon-gu, Seoul, 153-781 Korea.

Keywords: PCR, Mtcyt-b gene, Dorcas, Gazelle

INTRODUCTION

One of the most desert-adapted gazelles, dorcas gazelles may go their entire lives without drinking any water, obtaining all needed moisture from the plants which they eat [1]. They can withstand very high temperatures, although during hot weather they are primarily active at dawn, dusk, and throughout the night. Herds wander over large areas searching for food and tend to congregate in areas where recent rainfall has stimulated plant growth [1-4]. According to East [5], dorcas gazelle still ranges in Algeria, Burkina Faso, Chad, Djibouti, Egypt, Eritrea, Ethiopia, Libya, Mali, Mauritania, Morocco, Niger, Somalia, Sudan, Tunisia and Western Sahara; its occurrence in Nigeria is very doubtful, while it is considered extinct in Senegal. Its geographical distribution was acquired from Yom-Tov et al. [6].

The Polymerase Chain Reaction (PCR) is a scientific technique in molecular biology to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence, developed in 1993 by Mullis [7]. The method relies on thermal cycling, consisting of cycles of repeated heating and cooling of the reaction for DNA melting and enzymatic replication of the DNA. Primers containing sequences complementary to the target region along with a DNA polymerase are key components to enable selective and repeated amplification [8]. As PCR progresses, the DNA generated is itself used as a template for replication, setting in motion a chain reaction in which the DNA template is exponentially amplified. PCR can be extensively modified to perform a wide array of genetic manipulations [8].

MATERIALS AND METHODS

Blood samples were collected from different species of animals (Gazelles, sheep, goats, cows, camels, donkeys, horses and pigs) in blood container with Ethylene Diamine Tetra Acetic Acid (EDTA); these samples were used for DNA extraction. The extraction used by Qi-Agen commercial kit.

Selection of the primers for PCR

For detecting the mitochondrial cytochrome-b gene for

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gazelle we designed the primers by use bioedit software with accession number JN410257.1 of *Gazella dorcas* isolate from west 7 cytochrome-b (cytb) gene, complete cds; mitochondrial Tunisia from GenBank. The left primers included bases of the positive sense strand of the positive sense strand 5: CCT AGT TCT CAC ACT CCT AGT T. The right primers include bases of the complementary strand 5: GAG ACT ATT TTC AAT GGT ACT. The PCR product 421 bp and the primers were synthesized by World Meridian 10F, Gasan-dong, Geumcheon-gu, Seoul, 153-781 Korea.

Extraction of DNA from blood samples

For extraction of the DNA from blood samples we used commercial kit (QIAamp blood kit- QIAGEN Inc., Chatsworth, CA, USA) according to the manufacturer´s instructions. Briefly, 200 µl from the Lysing buffer (L.A) put in sterile eppendorf tube, then added 200 µl from the blood sample and added 10 µl from lyses enhancer, vortexed the tubes then incubated in water bath at 70°C for 10 min. Add 200 µl absolute alcohol, vortexed and incubated in water bath at 70°C for 2 min. Transferred all contents from the eppendorf tubes and put to QIA spin column. Spin at 8000 rpm for 2 min and discarded the deposit. Washed with 500 µl buffer one, vortexed and spin at 8000 rpm for 2 min. Changed the QIAamp column and washed with 500 µl buffer two, vortexed and spin at 12000 rpm for 3 min. Discarded the deposit and put the QIAamp column in eppendorf tube and add 200 µl from elution buffer and left for 1 min. Put in the spin for 8000 rpm for 2 min then discard the column and saved the eppendorf tube at freezing.

Tissues preparation

One dorcas gazelle slaughtered and took small part from different organs like: tongue, lung, heart, liver, kidney, spleen, muscles, lymph node, rumen, reticulum, omasum, intestine). The tissues were prepared from different organs by chopping to small pieces finally to form homogenous extract by scalpel, put every tissue in separate eppendorf tube, then add 300 µl of Distilled Water (D.W) and vortexed hardly, put the tubes in deep freezing for 10 min, thawing and vortexed. Repeated these steps 3 times, then centrifuged all samples at 300 rpm for 1 min. Aspirated 200 µl from supernatant for DNA extraction [9].

Cooked meat preparation

About 2 g of scapular muscles was boiled for 5 min, chopped finely to form homogenous extract, the whole volume was moved to an eppendorf tube and diluted with added 300 µl distilled water, vortexed hardly. The tube was kept into -20°C in deep freezer for 10 min, then thawed and vortxed, three times successively. All the samples were centrifuged at 300 rpm for 1 min. A volume of 200 µl of supernatant was aspirated to be used for DNA extraction [10].

DNA extraction from tissues and cooked meat

For extraction of DNA used the different organs of slaughtered gazelle and cooked meat. Commercial kit (QIAamp blood kit- QIAGEN Inc., Chatsworth, CA, USA) was used according to the manufacturer's instructions. Briefly, 200 µl from the Lysing buffer (L.A) were put in sterile eppendorf tube, then 200 µl of tissue lyses buffer were added, then, 200 µl from the supernatant sample were added, vortexed the tubes then incubated in water bath at 70°C for 10 min. A volume of 200 µl absolute alcohol were added, vortexed and incubated in water bath at 70°C for 2 min. The contents were transferred from the eppendorf tubes and put to QIA spin column. Spinning was performed at 8000 rpm for 2 min and the deposit was discarded. Washing was carried out with 500 µl buffer one, vortexed and centrifuged at 8000 rpm for 2 min. The QIAamp column was changed and washed with 500 µl buffer two, vortexed and centrifuged at 12000 rpm for 3 min. The deposit was discarded and put the QIAamp column in eppendorf tube and 200 µl from elution buffer were added and left for 1 min. Centrifugation was carried out at 8000 rpm for 2 min, then, the column was discarded and the eppendorf tube was kept at freezing degree.

Polymerase chain reaction (PCR)

Initially, the PCR conditions were optimized by varying the annealing temperature between 54-57°C on gradient thermo-cycler IQ-Cycler (BioRad) and the optimum annealing temperature which selected was 54°C. Prepared the PCR mixture containing 200 µl 10x PCR buffer, 100 µl of $MgCl_2$, 100 µl dNTPs complete the volume to 1500 µl by deionized distilled water (ddH_2O). Solved the primers: The left primer solved 348 µl, the total volume 100 pmol/µl to final concentration volume 348 µl × 2=696 pmol/µl. The right primer solved 276 µl, the total volume 100 pmol/µl to final concentration volume 276 µl × 2=552 pmol/ µl. 42 µl PCR mix taken and mixed with 2 µl primers, 1 µl Taq DNA polymerase 5 µl targets DNA from dorcas gazelle put in thermal-cycler tubes. All PCR amplification reaction were carried out in a final volume 50 µl .Vortex the mixture and adjust the thermal-cycler apparatus at the specific program according to melting temperature (T_m). The thermal cycling profiles as follows: at 95°C, followed by 40 cycles of 94°C for 1 min, 54°C for 30 s and 72°C for 45 s and final incubation at 72°C for 10 min. The total time of program was 2 h and 12 min. After PCR 10 µl were taken from the product with 3 µl from loading dye mixed and loaded onto gels of 1.0% Seakem agarose (FMC Bioproduct, Rockland Me) and electrophoresed. Put the gel onto ethidium bromide, the PCR products were easily identified under UV light.

Polymerase chain reaction specificity

For determining PCR specificity, 5 µl were taken from DNA extracted from (dorcas gazelle, cattle, sheep, goat, camel, pig, horse and donkey) and put each one in separate tube

with 45 µl from PCR mix, 2 µl primers and 1 µl Taq. All tubes were put in thermal cycler at specific program as follows: at 95°C, followed by 40 cycles of 94°C for 1 min, 54°C for 30 s and 72°C for 45 s and final incubation at 72°C for 10 min. The total time of program was 2 h and 12 min. Then 10 µl from the PCR products were taken and mixed with 3 µl from loading dye mixed and loaded onto gels of 1.0% Seakem agarose (FMC Bioproduct, Rockland Me) and electrophoresed. Put the gel onto ethidium bromide, the PCR products were easily identified under UV light.

RESULTS

The results of this study identified mtcyt-b gene in blood from 10 dorcas gazelles and show the sensitivity and specificity of the synthesized primers with target DNA from gazelle and other animals' species. The gazelle-specific 421 bp PCR product from blood samples were visualized under UV light shows **(Figure 1)**. PCR from DNA extracted from tissues of *Gazella dorcas* shows **(Figures 2 and 3)**. PCR from DNA extracted from cooked meat of *Gazella dorcas* shows **(Figure 4)**. Specificity of the PCR is showed in **Figure 5**.

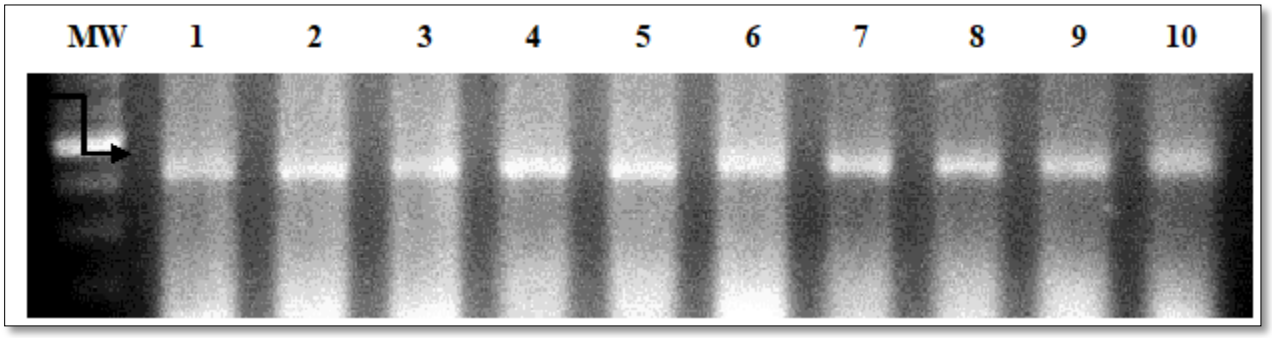

Figure 1. Detection of the 421 bp PCR product from dorcas gazelle blood samples.
Lane MW: Molecular weight marker; Lanes 1-10: Positive samples

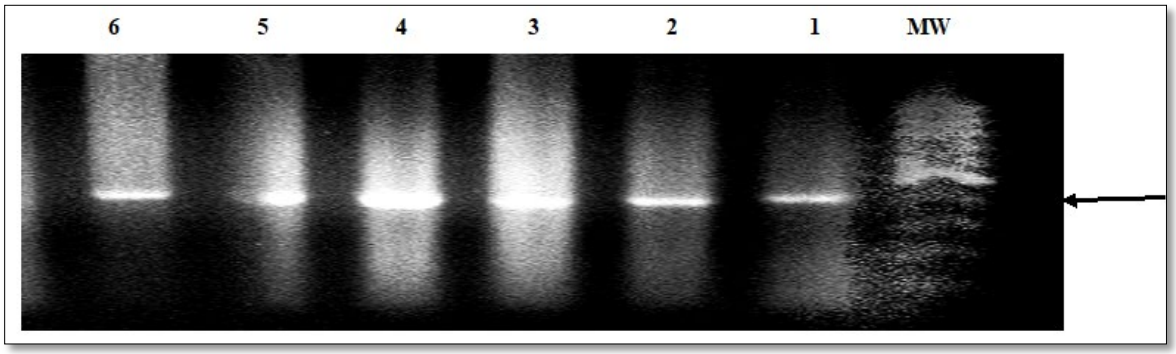

Figure 2. PCR from DNA extracted from tissues of *Gazella dorcas.*
PCR product: 421 bp for mitochondrial cytochrome-b gene from tissues. Lane MW: molecular weight marker. 1: Kidney, 2: Lung, 3: Liver, 4: Heart, 5: Lymph node, 6: Scapular muscle

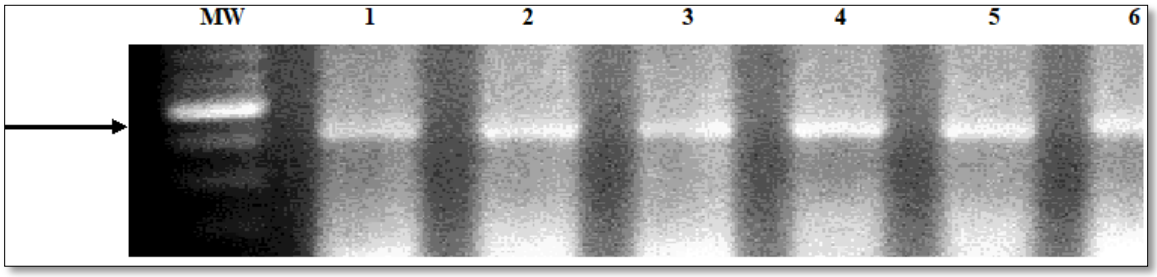

Figure 3. PCR from DNA extracted from tissues of *Gazella dorcas.*
PCR product: 421 bp. Lane MW: Molecular weight marker, 1: Rumen, 2: Omasum, 3: Reticulum, 4: Tongue, 5: Intestine. 6: Spleen

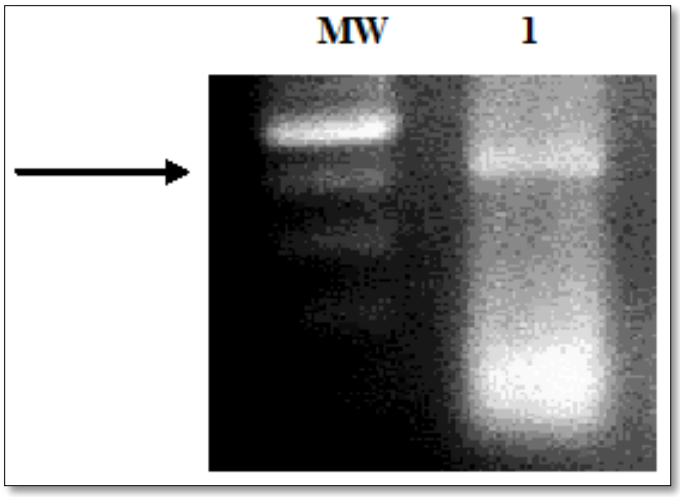

Figure 4. PCR from DNA extracted from cooked meat of *Gazella dorcas*.
PCR product: 421 bp for mitochondrial cytochrome-b gene. Lane MW: molecular weight marker, 1: cooked meat

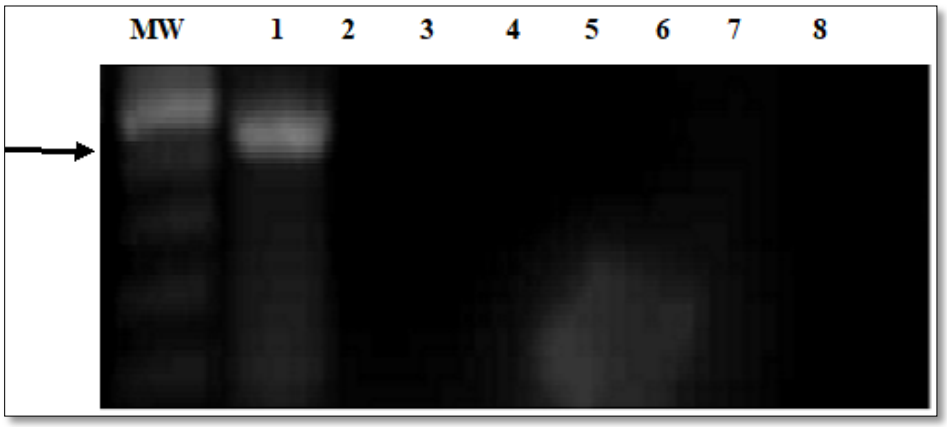

Figure 5. Specificity of the PCR.
Detection of the PCR product 421 bp from dorcas gazelle blood sample. Lane MW: Molecular weight marker (100 bp DNA ladder); Lane 1: positive sample for mytc-b gene from dorcas gazelle, from 2 to 8 were negative samples: cattle, sheep, goat, camel, pig, horse and donkey

DISCUSSION AND CONCLUSION

Lack of detailed information about threatened groups of animals can hamper conservation efforts [11,12]. Animal or meat species identification has been developed to address different concerns. Authentication of food ingredients is important for a consumer because of food fraud was spread. The traceability of meat component in food improves consumer's confidence in food products. The substitution of expensive meat with cheaper one is a major concern. For some consumer groups, such as vegetarians, the contamination of food with meat residue is strictly prohibited. Another good example of meat identification is the Halal food for the Muslim consumers, who are prohibited from consuming pork. In this study we used the tissues and cooked meat from *Gazella dorcas* to detect (mtcy-b gene) using PCR and this in accord with findings of various researchers [13-18].

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